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The Journal of Laboratory and Clinical Medicine

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VOL. XIV ST. LOUIS, MO., OCTOBER, 1928 NO. 1

CLINICAL AND EXPERIMENTAL

THE THERAPEUTIC USE OF BACTERIOPHAGE IN SUPPURATIVE CONDITIONS*

REPORT OF FIFTY CASES

BY THURMAN B. RICE, A.M., M.D., AND VERNE K. HARVEY, B.S.
INDIANAPOLIS, IND.

AS A LABORATORY phenomenon the bacteriophage is now an accepted fact though its exact nature is far from being definitely settled. As a therapeutic measure, however, there is much less unanimity in spite of the fact that d'Herelle has made large claims for it almost from the first. The following series of cases is reported in the hope that so important a matter may be brought into more general application if proved successful. We have used the bacteriophage in a somewhat different manner than is commonly recommended. The results have been gratifying in most of the fifty cases which have been treated.

The manner of preparing the bacteriophage filtrate is important, and we are of the opinion that most of the failures described by various writers and experimenters have been due to the inactivity of the strain of bacteriophage used for the particular strain of bacteria in the lesions under treatment. In each of the cases here described there was active, though not necessarily complete, lysis of the autogenous culture in vitro. (An exception to this rule is made in a few of the cases with very acute infections, as boils, which did not give time for an autogenous technic. In these cases material prepared for a similar case was used first, and then if time permitted before the cure was complete the autogenous preparation was used later.)

Our strains of bacteriophage have been obtained from various sources, but mostly they have originally been isolated from mixed sewage, and then "trained" to activity by being grown with the particular organism against which lytic action was desired. When a new case was taken, various strains of bacteriophage were tried with the autogenous culture, and the most active

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one chosen. In more recent work we have mixed a number of active strains and have added this mixture to the bacterial culture. Considerable activity has then usually been obtained at the first attempt. The culture medium that we have used has been either hormone broth or veal infusion broth with a hydrogen-ion concentration of 7.6. We have used Seitz filters instead of the Berkefeld candles, and have found them more satisfactory than the latter.

Our efforts have been confined to infections due to Staphylococcus aureus, Staphylococcus albus, Escherichia coli (Bacillus coli), and Pseudomonas aeruginosa (Bacillus pyocyaneus). Best results have been observed in those cases for which we have obtained the best lysis, and as a rule the poor results have been seen when the bacteriophage showed low ability in vitro. Early in the series we gave part of the material by subcutaneous injection but for the most part we have used the bacteriophage filtrate as a wet dressing applied directly to the lesion, or as an instillation into a sinus, an abscess cavity, or into the urinary bladder. In two cases the material was injected into an unopened abscess with unusually good results. Many of our cases have been dressed two or three times a day for weeks with the filtrate poured directly into the wound or impregnating the gauze in contact with the tissues. In this respect we have differed rather widely from the technic commonly used.

There are four possibilities to be considered in explaining our results:

1. That the good results are due to the destruction of the offending organisms by the bacteriophage according to the common interpretation of the Twort-d'Herelle action.

2. That the result is due to the antivirus action as described by Besredka. In every bacteriophage filtrate as commonly prepared there are, of course, the metabolic products of bacterial growth, which means that to that extent the filtrate is an antivirus preparation. We are conducting another series of experiments at the present time in which the bacteriophage is not being added to the cultures, and are getting results similar to those reported by Besredka and his followers. It may be that bacteriophage is also present in these preparations. This series will be reported later.

3. That the action is due to the presence of an unusually efficient and available form of the bacterial antigen which has been formed by the lysis of the causative organism by the bacteriophage.

4. Finally it is by no means impossible that the result is due to a combination of these various properties, or that indeed all are but different manifestations of the same thing. There is also the possibility that the broth itself exerts a beneficent effect.

The following cases represent all that we have treated with the exception of a few who died of their primary condition or left the hospital before there had been time for the effect of the therapy. It should be understood that early in our experiments we were given only cases that were considered to be quite hopeless, and that several of these patients died under circumstances which cast no reflections upon bacteriophage therapy. Some of the cases are of little value in this report, but are included in order that our poorest as

well as our best results may be evaluated. The cases are grouped according to the type of pathology encountered.

Boils.—In all cases of boils treated the organism was shown to be Staphylococcus aureus, and the bacteriophage strain used was active in vitro. The tubes were rarely cleared to the degree commonly observed when Bacillus coli was used, but active bacteriophage action was considered to be present if the culture showed the marked characteristics of a "rough" staphylococcus growth as compared with the control culture of the same organism without bacteriophage, i.e., when self-agglutination, more or less clearing, and a yellowish coloring of the broth were observed.

The following results with minor variations were observed in nearly all of the cases treated: (1) The boils were aborted when the filtrate was applied early in their development. (2) If already well developed they went through their evolution more rapidly than is commonly the case. (3) The pus coming from them was usually liquid, and when evacuated left a clean smooth crater. (4) Pain was usually promptly relieved, and the lesions showed diminished soreness after a few hours at the most. (5) The lesions were promptly healed with little induration, and there was rarely recurrence.

It is interesting to find Hauduroy, Camus and Dalsace referring to "a very marked diminution of pain, a rapid disappearance of erythema, the early softening of the induration, the reabsorption of the edema, and a rapid liquifaction of the purulent material" (close translation from the French, Presse med. Sept. 22, 1926) as following closely the use of the bacteriophage in the treatment of boils. We had observed the same phenomena exactly many times before reading their article.

The prompt relief from pain following this treatment requires special attention. It is so striking and often so complete that one who has not observed it may be pardoned for being skeptical. There are, however, numerous references in the literature to observations even more striking than those we are reporting. Several of these references are to the use of antivirus, but we are inclined to believe that the two phenomena are related, and we have observed the same effect after the use of both bacteriophage and antivirus filtrates.

We have repeatedly seen patients enter the laboratory guarding the seat of a boil or carbuncle in the most careful manner in order to avoid jars and bumps. After the application of a wet bacteriophage dressing for as short a time as ten or fifteen minutes, or more commonly after an hour or two, we have frequently been able to squeeze the lesion rather roughly without causing complaint. Many have remarked, entirely without suggestion from ourselves, that the pain and throbbing have stopped in a short time, and that muscular action which had before been unbearable had become easy.

CASE REPORTS, BOILS

CASE 7.—H. A. (City Dispensary), male, aged thirty-eight, had nine large boils on his left forearm. Bacteriophage prepared for another case of boils was used. The patient was given enough of the material to last for three days after which he was to return for the autogenous preparation. He did not return for three weeks having been "cured" by

the treatment. When he finally came back he had had a recurrence, and presented fifteen rather large boils and two carbuncles on the hands and forearms, except that there were none on the left forearm on the area previously treated. When the autogenous preparation previously made was applied there was immediate relief from pain and soreness, and a complete cure with no new lesions after eleven days. Urine examination showed large amounts of sugar, and there was clinical evidence of diabetes.

CASE 6.—Whar., a student nurse, had been having boils for months. At the time that treatment was begun she had a number of large and painful boils. There was complete cure in one week. One boil was injected with the filtrate and promptly retrogressed. The injection was of course very painful and for this reason wet dressings were mostly used. The patient has had a few boils since, but they have been smaller, less painful and mostly in locations not treated locally.

CASE 14.—Baby K. (Riley Hospital, No. 4312), male, aged five months. This child began at the age of three months to have boils. When first seen by us two months later, it was covered from head to foot with them, there being between 250 and 300 definite lesions of various sizes. Several of these were a centimeter or more in diameter, and a few were twice that size. They covered practically the entire body making the skin fiery red, and almost boardlike. The baby was haggard, thin, and whining constantly. It was unable to sleep and was running some temperature. Filtrate prepared for another case was first used while the autogenous preparation was coming on. The following day the child was better and more comfortable. After another day the improvement was marked, the child being able to sleep well, and to laugh and coo when awake. After eight days all but the large lesions were gone and the skin was soft. A few days later there was but one boil, and the child was gaining rapidly in weight and strength. The child was discharged completely cured and in excellent condition twenty-three days after our treatment began.

CASE 15.—V. W., a medical student, aged twenty-two, was subject to occasional boils. An unusually severe one came in the right nostril. He had been cautioned to avoid manipulation. It was necessary to take large doses of salicylates to keep down the pain, but even so he was pacing the floor unable to sleep, study, or attend classes. A pledget of absorbent cotton was saturated with the bacteriophage and inserted into the nostril, being replaced every half hour. Another similar pledget was placed over the tip of the nose. Absolutely all pain was gone after three and one-half hours. The next morning even the soreness was gone except for one little spot. During the second day the boil opened and about five drops of liquid pus was evacuated, and in forty-eight hours all signs of the boil had disappeared.

CASE 21.—Dr. O., a prominent surgeon had for six months been subject to large numbers of painful boils on the left forearm. He had tried "everything" and had gotten no relief. Bacteriophage suspended in a soft agar gel was given him for local application. He reported that pain was relieved, that the boils were less sore, and were smaller. After about two months of treatment he was free of them. Treatment was discontinued for about a month and at the end of this time there was recurrence (six boils).

CASE 23.—C. K., male, aged twenty-one, a patient of Dr. H. This man had a pimple under his left arm. He applied a poultice to it, and then shaved the axilla to remove the matted hair and poultice material. A large carbuncle-like lesion developed in the axilla and surrounding region as a result of his nicking the skin with the razor. The patient was in serious condition, had high fever, and was delirious. The usual means of treatment were giving no improvement. Bacteriophage was applied December 22. There was immediate improvement, and the case was dismissed cured on January 2. The attending surgeon's words describing the case are as follows: "Induration receded. The axilla is in such condition as is usual only months after such an inflammatory process. Full credit is given to the bacteriophage."

CASE 30.—F. L. (Industrial Clinic), male, aged twenty. This man was a worker in silica, and probably as a result had a great number of large and painful boils, about fifty, on each forearm. There were also several on the legs and a few on the abdomen. After three treatments the man was so improved that he did not come back, and was not seen for a week. At this time he was much improved, being practically well except for two or three of the larger lesions which were healing. There were no new boils.

CASE 51.—Mr. B., medical student, had a large boil or small carbuncle on the back of his neck. There was prompt relief from pain upon application of the bacteriophage in the form of a wet dressing. It improved rapidly and was healed. About ten days later he came back with another unusually deep lesion in the same location. This time the bacteriophage was ineffectual, probably because the seat of the trouble was deep in a thick layer of fat and subcutaneous tissue. We proposed to inject the filtrate into it with a needle, but permission was not granted.

CASE 35.—J. S., male, aged seventeen, has had many large boils on his face in recent months, and also a marked pustular acne. His general physical condition was found to be bad, and his urine showed a large amount of albumin, three-plus, on several occasions. He was sent to a genitourinary specialist who in turn sent him back to us thinking that the kidney condition was secondary to the infection of his face. With specific wet dressings and bacteriophage suspended in soft agar applied to the face for a period of six weeks the face is now nearly clear, there have been no more boils, and there is barely a trace of albumin found occasionally in the urine. His general health appears to have improved.*

CASE 36.—Dr. K. had a carbuncle on the back of his neck. Wet dressings of bacteriophage filtrate were applied. There was relief of pain after about ten minutes. This treatment was witnessed by four other physicians who were skeptical, but who will now vouch for the above statement. The core came out two days later leaving a clean crater, and the boil healed promptly without further pain.

CASE 41.—J. K., male, aged fifty-five, had two boils in the axilla, one of them well developed and the other only started. There was immediate relief, and a complete cure in three days.

CASE 43.—A. D., female, aged eighteen, had a painful stye. There was prompt relief from pain, the pus was evacuated after a few hours, and a condition that gave promise of being severe was well in less than two days.

CASE 39.—Mrs. S., aged thirty-one, frequently has had superficial boil-like lesions on the face. They were painful and unsightly, leaving a red blotch which would persist for a long time. Bacteriophage wet dressings were followed by prompt healing, and when applied early seemed to abort the infection. She has had none for several weeks.

Abscesses.—The treatment of abscesses has been much like that of boils except that the bacteriophage filtrate has been injected into the opened or unopened abscess, or used to saturate gauze dressings which were then packed into the cavity. Mixed infections were treated with mixed bacteriophage filtrates prepared separately at first, but later it was found that it was more convenient to grow the material in mixed culture, and prepare the material by one manipulation. Reisolation at short intervals was always practiced when mixed cultures were used or when the case was found to be resistant.

*Since the above was written he has had a recurrence of the acne and has taken other treatment for it under a skin specialist.

CASE REPORTS

CASE 1.—C. S. (Long Hospital, 23262), male, aged fifty-five. This was the first case treated, and as a result of inexperience not as well handled as have been subsequent cases. This patient was selected because his condition was hopeless from the first, and for the reason we were more inclined to try the new method of treatment. His primary injury was a crushed pelvis resulting from an accident in which a tree fell across his body. The bladder was ruptured and there was extravasated urine throughout the pelvis. Large amounts of foul pus containing Bacillus coli were pouring from various sinuses, and from the bladder. Several weeks had passed since the accident and the patient was at the point of death when treatment was begun. Almost his entire back was covered with bed sores. There was an immediate change in the nature and the amount of the pus; the odor disappeared almost entirely, and after a few days there was evidence of new granulation tissue; the openings of the sinuses seemed more healthy. The man died as a result of the extension of the bed sore to the spinal canal. Autopsy showed a healthy condition of much of the tissue lining the various abscess cavities of the pelvis.

CASE 2.—Chad. (Riley Hospital, 3225), female, aged five. The child had had mastoiditis. For six weeks there had been no hope of recovery, the temperature was about 104 to 105°, she was comatose, and large amounts of extremely offensive pus were pouring out. Diagnosis of brain abscess had been made. Staphylococcus aureus and Bacillus pyocyaneus were isolated. Active bacteriophage action was obtained against both organisms, we had been working on the development of an active bacteriophage for this case for several days before being given the opportunity to use it. Her condition appeared immediately to improve after the filtrate was used; the temperature went down; odor and pus disappeared entirely; coma was relieved so that the child could talk and ask questions. The marked improvement continued for four days when there was sudden relapse and later death. Autopsy revealed a cerebral abscess involving nearly the entire left hemisphere, and an extreme suppurative pansinusitis.

CASE 8.—Bur. (Riley Hospital, 4012), male aged sixteen, was thought to have an unknown focus of infection which caused him to develop numerous large abscesses in various parts of the body at intervals of a week or less. When opened these abscesses would drain for weeks before healing. Staphylococcus aureus was isolated from the pus. Active bacteriophage was obtained and injected into two unopened abscesses, one in the left breast and the other in the left thigh. A similar lesion in the right breast had drained for three weeks after being opened, while the one injected with bacteriophage was dry from the first after being opened, and it healed much more quickly. The abscess in the thigh healed much more promptly than had been anticipated, and no other lesions developed. The boy was discharged well.

CASE 31.—R. S. (St. Francis Hospital), male, aged thirty, had a discharging appendiceal abscess that would not close. It had been dressed daily for three weeks, and there was still a considerable amount of pus. Staphylococcus aureus was the organism. The pus stopped, and prompt healing followed the use of the filtrate prepared for another case.

CASE 40.—H. (St. Francis Hospital). Following a nephrectomy operation the wound became infected with Staphylococcus aureus, and there was much pus. After a few days of treatment with the bacteriophage the wound healed completely.

CASE 34.—Mrs. H. (St. Francis Hospital), aged thirty-seven. This patient, who had been brought into the hospital six weeks previously in a delirious condition, had an abscess between the skin and the sacrum that was *eleven* inches across, and from this there poured 250 to 350 c.c. of putrid pus daily; she also had a left pyosalpynx, marked pyelitis, cystitis, pus, blood, and casts in the urine in large amounts, a badly degenerated myocardium, hemoglobin of 40 per cent, and red cell less than 2,000,000, in spite of three rather recent transfusions, and was running a septic temperature.

An unusually active bacteriophage was obtained against strains of Bacillus coli and staphylococcus isolated and reisolated from the abscess. The odor of the pus was mostly gone after one day's treatment, the abscess was dry after a week, and her general condition was much improved; the mental condition improved so that the patient became rational; the temperature fell almost at once to 100 to 100.6°; and she began to eat better and to take interest. About this time an abscess developed on the right arm. Bacteriophage was injected into it before it was opened, three injections of about 2 c.c. each. An incision was then made (one and a quarter inches long) and an ounce and a half of very liquid pus was evacuated. After the first drainage there was never any pus from this abscess, and five days later the incision was completely healed, and the abscess cavity closed without redness, soreness, or induration, and this in spite of the fact that three clean transfusion wounds were still open after weeks of time. The filtrate was subsequently applied to these wounds and they healed in a few days. The large abscess over the sacrum about this time began to close in quite rapidly. One month after beginning the treatment this abscess was about one-third its former size, and the patient was gaining weight at the rate of nearly a half pound a day. Her blood condition was much improved in spite of the fact that there were no more transfusions. Twenty-one days after beginning the treatment she was afebrile and remained so. Ninety days after entering the hospital, and forty-five days after the bacteriophage was first used she went home well. Several good physicians had considered recovery impossible. This is a conservative statement of a case that can only be appreciated by having been seen.

CASE 2.—F. D. (Riley Hospital, 3471), female, aged five, had a subphrenic abscess which had been drained but was not closing properly. Large amounts of thick pus continued to drain. Specific bacteriophage was instilled into the cavity, and the pus immediately decreased. The bacteriophage was discontinued after a few days and the pus returned. It was then resumed; the pus stopped and the cavity closed rather rapidly. The child was sent home well.

Ulcers.—Ulcers of various sorts have been treated very much as have been abscesses. The most surprising results have been those in connection with the treatment of bed sores. We had supposed that these were atrophic ulcers due to pressure and devitalization of tissue rather than bacteria, but it should be remembered that there is always infection in them, the commonest significant organisms being Bacillus coli and staphylococcus. The fact that these cases may be improved by this treatment was discovered quite accidentally when it was noticed that the bed sores of the first case treated began to improve when the filtrate was instilled into the bladder in the treatment of the coincident cystitis. It was quite surprising to see large bed sores become clean and start to heal; to see the pus checked, and to find that the odor had gone. So far as we can find this is the only reference to the treatment of bed sores with bacteriophage in medical literature.

CASE REPORTS

CASE 5.—A. C. (Long Hospital, 24234), male, aged sixty-nine, had had hypertrophy of the prostate, residual urine, severe cystitis. A suprapubic cystotomy had been done before coming to the hospital; a periurethral abscess had developed; he was running a septic temperature, and was very thin and weak. Two large bed sores developed; one over the sacrum was about six inches in diameter and was deep, the other on the leg was about three by five inches. His condition was such that death seemed imminent. Bacteriophage dressings of the sores were begun, and the same material instilled into the bladder. The temperature fell to normal the next day, and remained there. The bed sores began to

clean themselves, and were dry and odorless at the end of three days. At the end of a week there were healthy granulations, and new epithelium was spreading from the edges. After a month the bed sores were practically healed, the cystitis was much better, and the surgeon was ready to remove the prostate. The patient, however, refused further surgery and went home in good condition.

CASE 13.—K. B. (City Hospital) was a colored boy, aged fifteen, with a complete transverse ascending lesion of the cord. There was complete sensory and motor paralysis from the hips down, and there was incontinence of bladder and bowel. The trouble was of several months' duration and the legs were covered with large and small bed sores which were extremely foul. There was of course no hope of curing the primary condition, but it was thought that we might be able to control the pus and odor thereby making the case easier to handle. A fairly active bacteriophage for mixed cultures of Bacillus coli, Staphylococcus aureus, and Bacillus pyocyaneus was obtained. Immediately after the treatment began the odor stopped almost entirely, the sores became clean and healthy in appearance. After a week there was evidenc of healthy granulation tissue. The boy complained that the ulcers next to the sensitive skin were itching and smarting, interpreted as an evidence of healing. Five weeks after the treatment began the patient died due to the progress of the primary lesion causing an ascending paralysis. At the time of his death there were ulcers two inches in diameter which had completely healed and most of the other larger sores had a rim of about one inch of new epithelium about them, were clean, and had good granulations in the base.

CASE 27.—I. P. (Long Hospital, 25280), male, aged eighty-five, had had a cancer of the upper lip which had been removed surgically and also treated with radium. There was an ugly pus-filled ulcer on the upper lip. Local applications of an autogenous anti-staphylococcus bacteriophage filtrate caused relief of pain and soreness, and soon cleaned the ulcer. It healed in a few days except for one place about one centimeter in diameter, which was considered to be a radium burn. The patient after several weeks developed erysipelas. This condition was improving when pneumonia set in and the patient died. Autopsy examination revealed the fact that the ulcer had at last entirely healed.

CASE 37.—T. (Long Hospital, 25632), male, aged fifty-six, had had a cancer of the lip removed months before. Metastasis had involved a neighboring lymph gland, and this was removed with the cautery knife leaving a denuded area about eight by ten centimeters. This wound became infected with Staphylococcus aureus and made a rather severe sore. To answer the criticism of a colleague, simple broth of the kind that we used for the making of the bacteriophage was used at first. There was an increase in the amount of pus under this treatment after a week. Bacteriophage filtrate was then used and the denuded surface became clean in twenty-four hours. After a few days there was healthy granulation tissue over the entire surface. The patient was sent home at this time. Three weeks later he returned with the wound doing well and about half its former size.

Acne.—Early in our experience with the therapeutic use of bacteriophage we noticed a student with an extremely bad case of pustular acne. We suggested trying the use of bacteriophage, but with no great assurance of being able to give help. This is the first case reviewed below. Our results in the treatment of acne have been peculiar in that we have had better success in the bad than in the mild cases. As soon as the active inflammatory stage of the condition has been cleared we have usually recommended x-ray in the hope that the oil glands of the skin may be permanently checked in their excessive activity, thereby removing the principal cause of the condition. The organism isolated has in nearly every case been Staphylococcus albus, rarely

aureus. The attempt has not been made to isolate the "acne bacillus" as we have been inclined to the belief that it is only a secondary invader and not the etiologic agent.

CASE REPORTS

CASE 9.—B., a medical student, aged twenty years. This was a severe case of pustular acne. The lesions were not deep, and there was no permanent scarring. There were about thirty-five active pustules on the face when we began; the face was sore, indurated and beefy red. Active bacteriophage action was obtained against the organism. The filtrate was made into a soft agar which was to be rubbed into the face twice a day after opening the pustules, then cleaning the face with alcohol, and finally washing with strong soap and hot water. In as much as this preliminary treatment had been used energetically for years without results it cannot be said that this was the cause of the improvement. There was a marked change in the space of three days. After three weeks there was scarcely an active lesion, and after six weeks the skin was soft and smooth and has remained so.

CASE 10.—G. J., male, aged eighteen, had a bad case of acne with deep pits and much induration. He had lost his job because of the condition of his face and could find no other employment for the same reason. His acne was of four years' duration, was getting worse, and had resisted every sort of treatment including x-ray. Treatment as in the case above was instituted. At the time that we began, his face was red, sore, and hard; very little pus could be expressed from the lesions. After a week the face was much softer and about three cubic centimeters of pus was expressed from the various lesions. A week still later the face was even softer, but very little pus could be obtained. After this there was little evidence of active inflammation though the face was, of course, badly scarred. His appearance was, however, greatly improved, and he was able to get a job. Since this time he has been unable to come to the laboratory, and we have not been able to keep in contact with the case.

CASE 11.—P. K. L., a medical student, aged twenty-one, had had severe acne for years. His face was badly scarred, pitted, indurated, and quite sore. Treatment as above described gave excellent results. The face became much softer, and it was possible to get a large amount of inspissated pus from the old lesions deep in the skin. No new pustules have developed since the treatment was begun. The pits and some of the nodules have remained. He has had several x-ray treatments since stopping our treatment and is now much improved in appearance.

CASE 8.—Bur. (Riley Hospital, 4012). The healing of abscesses in this case has already been described. The patient also had a marked pustular acne which entirely disappeared in the space of three weeks after we began using on his face some of the same filtrate that was used for the abscesses.

CASE 12.—M. F., male, aged nineteen, a strong athletic type with a marked pustular acne on chin, lips, and nose. There is no scarring. Relief came slowly, but finally all lesions were healed under the bacteriophage treatment. The use of the agar containing the active principles was then discontinued and the pimples began to reappear. Treatment was resumed and they again disappeared. X-ray was recommended to check the excessive oiliness of the skin.

CASE 16.—G. K., female, aged eighteen. This was a mild case. After two months the face is clear, but the case was so mild that the result is inconclusive.

CASE 17.—K. L., male, aged seventeen. This was a moderate case in a youth in excellent health. The case is still under treatment. Progress indicates that we may expect good results, but that it will be slow.

CASE 19.—J. H., male, aged twenty. This is a moderate case of six months' duration. The youth is away at college, and we have not seen him recently, but good reports have been obtained.

CASE 22.—Card. (Long Hospital, 25582), male, aged twenty-one. This was a rather marked case in a patient in the hospital for an appendectomy. He was sent home eight days after we began treatment, and at that time was well of acne except for the red blotches where the pustules had been. No new ones had developed.

CASE 25.—F. F., male, aged twenty-three. This was a moderate case with scarring. There is marked improvement. At one time three boils complicated the picture. Staphylococcus aureus was obtained from these boils. When bacteriophage active for both Stalphylococcus aureus and albus was used we got excellent results.

CASE 26.—M. B., a young woman with very slightly active lesions, but considerable scarring. She insisted on using bacteriophage though we told her it would probably do no good in her case. She thinks there is less induration, and active inflammation since using the preparation, but of this we cannot be sure.

CASE 33.—Ne., a student nurse with a moderate case of acne is still under treatment with little improvement. Immediately after the bacteriophage was first used there was apparent help, but as soon as she stops using it the pimples return about as they were at first.

CASE 38.—F. N. This was a severe case in a man of twenty-two. He was very recently discharged completely cured of active lesions. His skin is excessively oily and x-ray is recommended.

CASE 44.—C. F. This was a severe case in a boy of sixteen. He was much improved after the first week's treatment, and has not been seen since.

Urinary Infections.—Among the first attempts made to use bacteriophage therapeutically, were several cases of urinary infections due to Bacillus coli. We have treated no patients for cystitis alone, but in several of our other cases we have seen good effects come from its use. The filtrate can be instilled into the bladder as there seems to be no bad effects from such treatment. We have not seen the extremely rapid results reported by certain authorities, but have not failed to observe marked improvement in every case in which an active bacteriophage has been injected into the bladder of patients with Bacillus coli infection.

Cases 1, 5, and 34 are instructive in this connection. An interesting incident in connection with a case for which we were preparing an autogenous bacteriophage should be recounted here. We were ready to send the material when the physician in charge notified us that it was not necessary as the woman had quite suddenly greatly improved, and was practically well of her cystitis. We asked for a sample of her urine and found that it contained a strain of bacteriophage very active for the strain of Bacillus coli previously isolated from her urine. In this case the woman had evidently developed her own bacteriophage, and this was, we believe, responsible for the rapid change in her condition. This woman had other relapses following this, however, and also other rapid improvement periods. We have been unable to study her urine since as she lives at a distance from the laboratory.

Sewage is an excellent source of highly active Bacillus coli bacteriophage and little difficulty is experienced in preparing it from this source. Those who wish experience with this most interesting phenomenon may well begin with sewage as a source of Bacillus coli bacteriophage.

MISCELLANEOUS CASES

CASE 18.—S. D., a physician, aged fifty-nine, with inoperable cancer of the bladder of long standing. The growth had obstructed the return circulation of the penis and as a result a painful balanitis had developed requiring the use of over a grain of morphine daily to make life tolerable. There was imminent danger of moist gangrene. A pure culture of Staphylococcus aureus was obtained and an active bacteriophage filtrate prepared. There was prompt relief from pain and complete healing of the balantis in about a week in spite of the fact that the primary condition was progressively becoming worse. At the time the treatment was begun the balanitis was of eight months' duration and becoming worse. The treatment was stopped, and the ulcers returned after about three weeks.

CASE 32.—H. (Long Hospital, 25249), female, aged twenty-two. About a year before this case was admitted to the hospital she had had an operation for tuberculous peritonitis and had been left with a fecal fistula. At the time that we began the treatment there was a large ulcerated area (12 by 15 cm.) about the mouth of the fistula. The edges were deeply undermined in several places, as much as ten centimeters in one direction. Fecal matter in large amounts came into the wound constantly. There were large amounts of pus and it was necessary to dress the wound three times a day. The patient was getting worse, was extremely emaciated, and subject to delirious attacks warranting the diagnosis of toxic psychosis. When it was decided to send her home to die, we began to treat the infected sinus with bacteriophage and she was retained in the hospital for this special treatment. The amount of pus was soon reduced, the odor was less offensive, the wound became cleaner, and good granulation tissue developed at the base and sides. The opening in the skin is much smaller than it was when we began, the undermining is now no more than 2.5 cm. at the deepest place and her general condition is better. At one time there had been no feces in the wound for a month when a mistake in the use of ultra violet light (probably) greatly stimulated her causing her to become violently delirious, and causing her to tear the fistula open again. Feces again came into the wound, but has not been seen in the last two weeks. No attempt has been made to treat the tuberculosis except that good general care has been given, and intravenous glucose has been used about twice a week much of the time. Light was used twice and seemed to make her worse. The case has been under treatmnt for twelve weeks and is still a very grave one. There can be no doubt that there has been marked improvement in the wound and in the patient's general condition during this time, and this in spite of the fact that she had been going down hill for a year under all other methods of therapy.

CASE 24.—Inf. R. (St. Francis Hospital), female, aged fourteen days. On the tenth day of life this child developed impetigo over a considerable portion of the body. Anti-staphylococcus bacteriophage was used on a part of the lesions while the others were dressed with ammoniated mercury ointment. Two days later all the lesions were improved but those treated with bacteriophage were more improved. This treatment was then used on all of the lesions and they were completely well in two more days. This result was better than had been commonly attained in such cases in that hospital.

CASE 4.—M. S. (Riley Hospital, 3567), female, aged seven. This child had an osteomyelitis of the os calcis. The bone had been removed, but the entire foot was at the point of becoming gangrenous. Amputation was seriously considered. There was prompt improvement and recovery when the filtrate was used as an irrigation and a wet dressing.

CASE 49.—P. B. (Long Hospital, 25857), male, aged twenty-six. This patient had had a discharging sinus in the region of the left anterior superior spine of the ilium for seven years. The bone was thought to be involved, but exactly in what manner was unknown. There was immediate relief from pain, tenderness, and soreness when the irrigations were begun, and the patient was sent home well after about three weeks' treatment.

SUMMARY

1. Bacteriophage filtrates active for autogenous cultures have been used in a series of fifty suppurative conditions and have been found to be highly effective against Staphylococcus aureus and albus, Bacillus coli, and Bacillus pyocyaneus.

2. They have been found most effective when used as a wet dressing or when instilled into a cavity.

3. There is considerable evidence that stock cultures of the bacteriophage may be used with profit when there is not time or facility for the preparation of the autogenous product, or while it is in preparation. This is particularly true of the staphylococcus preparations.

4. Those who have seen the results in actual cases are invariably enthusiastic and are convinced that the method has much merit. At least it offers promise and should be thoroughly investigated.

REFERENCES

[1]D'Herelle: The Bacteriophage and Its Behavior, Williams and Wilkins, 1927.
[2]Dutton: Jour. Infect. Dis., 1926, xxxix, 48.
[3]Shartzman: Jour. Exper. Med., 1927, xlvi, 497.
[4]Besredka: Local Immunization, Williams and Wilkins, 1927.
[5]Bourdenko: Ann. d l'Inst. Pasteur, 1926, xl, 232.
[6]Carrere: Ann. de l'Inst. Pasteur, 1925, xxxix, 67.
[7]Epstein: Wien. klin. Wehnschr., 1927, xl, 80.
[8]Ibid: Wien. klin. Wehnschr., 1927, xl, 601.
[9]Gloukhoff: Ann. de l'Inst. Pasteur., 1927, xli, 189.
[10]Lehndorff and Brumlik: Wien. klin. Wehnschr., 1927, xl, 483.
[11]Nicolas: Ann. de l'Inst. Pasteur., 1926, xl, 1073.
[12]Nikolaewa: Ann. de l'Inst. Pasteur., xl, 1926, 869.
[13]Hayes: Laryngoscope, 1926, xxxvi, 812.
[14]Arnold and Weiss: JOUR. LAB. AND CLIN. MED., 1926, xii, 20.
[15]Bazy: Compt. rend. Soc. de biol., 1925, xcii, 485 (Abst. Jour. Am. Med. Assn., lxxxiv, 1389).
[16]Rice: Jour. Indiana Med. Assn., 1928, xxi, 93.
[16a]Friedlander and Toony: Jour. Exper. Med., 1925, xlii, 663.
[17]Besredka: Ars Medici, 1927, v, 511.
[18]Hauduroy, Camus and Dalsace: Presse med., September 22, 1926.
[19]Besredka: Local Immunization, 1927, Chapter II.
[20]Bruynoghe and Maisin: Compt. rend. Soc. de biol., 1926, lxxxv, 1120.
[21]Callow: Jour. Infect. Dis., 1927, xli, 124.
[22]Gratia: Compt. rend. Soc. de biol., 1924, lxxxvi, 276.
[23]Becherich and Hauduroy: Jour. Bacteriol., 1923, viii, 163.
[24]Larkum: Jour. Michigan Med. Soc., 1928, xxvii, 106.
[25]Gougerot and Peyre: Compt. rend. Soc. de biol., July 12, 1924.
[26]Nelson: Jour. Immunol., 1928, xv, 43.
[27]McKinley: JOUR. LAB. AND CLIN. MED., 1923, viii, 311.
[28]Ibid: Arch. Int. Med., 1923, xxxii, 899.
[29]Dalsace: Jour. d'Urol., 1926, xxi, 123.
[30]Frisch: Wien. klin. Wehnschr., 1925, xxxviii, 839 (Abst. Jour. Am. Med. Assn., lxxxv, 861).
[31]Munter and Boenheim: Ztschr. f. Kinderh., 1926, xxxix, 388 (Abst. Jour. Am. Med. Assn., lxxxv, 78).
[32]Larkum: Jour. Bacteriol., 1925, xii, 223.
[33]Ibid: Jour. Bacteriol., 1926, xii, 203-240.
[34]Zdansky: Wien. Arch. f. inn. Med., 1925, xi, 533 (Abst. Jour. Am. Med. Assn., lxxxvi, 317).
[35]Ravina: Presse Med., May 1, 1926, 548.

SOME COLOR REACTIONS OF MORPHINE AND ITS DERIVATIVES ON HEATING IN CONCENTRATED SULPHURIC ACID SOLUTION*

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INTRODUCTORY REMARKS

THE present paper, though complete in itself, is a continuation of the previous one entitled "Some New and Improved Tests for Morphine and Related Alkaloids." In that paper, among other things, there were described some reactions obtainable after heating morphine, or one of its derivatives, in pure concentrated sulphuric acid to various temperatures below that at which a color develops in the sulphuric acid solution itself, the temperature at which the sulphuric acid color test begins. The more important points in this connection are mentioned in article VI of the present paper, "Nature of the Reactions."

No attempt is here made to discuss the reactions of any alkaloids other than morphine, heroine, codeine, and dionine. The investigation of these tests requires pure substances, for with heating in concentrated sulphuric acid other organic matter if present might char and spoil the reaction. For some of the tests, the alkaloid must also be free from chloride or bromide. Time and opportunity have been lacking, thus far, for the trial of many alkaloids besides those mentioned. But indications are that all these tests are specific for morphine, and its most closely related derivatives. Substances giving similar reactions, if any exist, are no doubt phenols rather than nonphenolic alkaloids.

The exact amount of morphine required for a test is not stated in the descriptions. About as much should be taken as is ordinarily used in any well-known test of the same general character, for instance, Pellagri's test. References to "large" or "small" amounts of morphine are to be interpreted with this in mind.

I have attempted to make the descriptions brief, and at the same time mention all effects of any importance. In general, amyl alcohol, chloroform, ether, and benzol have been tried as solvents for each colored product, not only for extraction from the dilute sulphuric acid solution, but also from solutions to which ammonium acetate, sodium carbonate or bicarbonate, and ammonia had been added. It may be mentioned here that amyl alcohol will always extract the color if anything will.

It is not possible to give the temperature for a change with great exactness, because a change which occurs almost immediately at a certain temperature will take place gradually at lower temperatures. In the descriptions the temperatures stated were obtained, as a rule, by placing the test tubes in the

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bath at about room temperature, and heating gradually, but fairly rapidly, until the desired temperature was reached. A different method is to be followed only when it is stated that the test tube may be stood in the boiling water-bath for a certain length of time.

Objection may be made to the names I have assigned to these tests. Presumably it is actually arsenic acid which takes part in the arsenate test and chromic acid in the chromate test. However the name "arsenate test" seems to be well established, so I have adhered to it, and named the other tests to correspond. In the "halide-oxidation test" it is solid chloride or bromide (of sodium) that is added, but apparently free chlorine or bromine that actually reacts with the morphine.

None of these tests should displace any from a list of the 10 or 12 best tests for morphine, but they are not without value, as well as interest. The arsenate test, regulated by the thermometer and properly understood, is an excellent qualitative test and the same may be said of the perchlorate test. The new halide-oxidation test should prove even more useful.

MATERIALS USED

The alkaloids used in investigating these tests, with the source of each, were as follows:

Morphine hydrochloride—from samples.

Morphine sulphate—Mallinckrodt.

Morphine (free alkaloid)—prepared from the hydrochloride by precipitation with ammonia.

Codeine sulphate—Mallinckrodt.

Codeine (free alkaloid)—N. Y. Quinine and Chemical Works.

Heroine hydrochloride—Mallinckrodt.

Heroine (free alkaloid)—prepared from the hydrochloride by precipitation with ammonia.

Dionine (hydrochloride)—Merck.

Ethyl-morphine hydrochloride—Powers-Weightman-Rosengarten Co.

Dionine (free alkaloid)—prepared from the Ethyl-morphine hydrochloride (P.-W.-R. Co.) by precipitation with ammonia.

The sulphuric acid used was the C.P. acid, 95 per cent H_2SO_4, manufactured by the General Chemical Company. The analysis of this acid, as given on the label, was included in my previous article, referred to above. The U.S.P. sulphuric acid manufactured by the Hirsch Laboratories was also tried in some tests and gave exactly the same results.

I. THE SULPHURIC ACID COLOR TEST

When morphine is dissolved in pure concentrated sulphuric acid, and the solution heated, little or no color is developed for some time. In fact, the only color appearing below 130° or 140° C., if the acid and alkaloid are both pure, will be a slight violaceous or reddish color, doubtless due to a little oxidation by atmospheric oxygen. At about 140° or 150° C., however, with very little morphine, a dark olive-green color develops.

That morphine gives a color test in this way is, of course, well known. The color on dilution and on addition of ammonia have also been noted, but the colors extracted by amyl alcohol seem to have escaped attention hitherto. The test is as follows:

Put a little dry morphine or its salt in a clean, dry test tube, and dissolve it in a little pure concentrated H_2SO_4. Stand the tube in a suitable bath (say of phosphoric acid), provided with a thermometer, and heat. At about 140° to 150°C a dark olive-green color develops. Continue heating to about 175°C. The solution is then green-black. Cool; dilute with a few c.c. of water. The aqueous solution is dark blue-green, and a dark precipitate falls if it is allowed to stand. Divide this solution into two portions. To one portion add about 0.7 c.c. amyl alcohol and shake. The amyl alcohol readily extracts a deep, slightly purplish, blue color (ether, chloroform, and benzol extract little or no color). To the other portion add concentrated NH_4OH until alkaline. It becomes clear green. At once add a little amyl alcohol and shake. It extracts a bright green color. The color remaining in the aqueous layer soon changes from green to brown, and the green in the amyl alcohol also changes to brown, more slowly.

Heroine, codeine, and dionine give this same reaction. Even in the presence of impurities which char and spoil the other colors it is possible, sometimes at least, to get good blue and green colors in the amyl alcohol extracts.

If the sulphuric acid solution is cooled and diluted after heating only to 160° C., amyl alcohol extracts a purple rather than a deep blue color. The other colors are about the same, but not so strong, as when the heating has been carried to 175°.

The colors obtained in this test should be compared with those obtained in the arsenate and chromate tests at similar temperatures.

II. THE ARSENATE TEST

Several authorities, for instance Allen's "Commercial Organic Analysis," and Fresenius-Wells' "Qualitative Chemical Analysis," describe a color test given by morphine on heating in sulphuric acid containing a little sodium or potassium arsenate. These descriptions, without exception so far as I have found, are incomplete, fail to recognize the influence of chloride if present, state no temperatures, and in short are so misleading as to be almost worse than useless. They usually say also that codeine gives the same or a similar test.

The colors developed in the sulphuric acid solution constitute a test; and if we consider separately the colors extracted by organic solvents, then there are at least two other distinct tests in the arsenate reaction, depending on the temperatures to which the solutions are heated. Heating to 130° C. provides one set of colors in solvents, heating to 175° an entirely different one. Chloride, which may be present as morphine hydrochloride, is capable of changing the reaction completely. Bromide changes it even more readily. With codeine, the color changes in the sulphuric acid solution are entirely different from those produced by morphine.

Morphine.—First Arsenate Test: Color changes in the sulphuric acid solution.

Put a little morphine in the form of the free alkaloid or the sulphate, in a clean dry test tube, and dissolve it in a little pure concentrated H_2SO_4 (say about 8 drops). Add 4 drops of concentrated H_2SO_4 containing 5 gm. KH_2AsO_4 per 100 c.c. (Not much Arsenate is required for a strong reaction, but, for temperatures below 150°C. or thereabouts, an excess does no harm.) When the arsenate is added as soon as the morphine is dissolved, a blue color develops at once, if a fair amount of morphine is used. When very little morphine is used this blue color will not be noticed, as it is not strong. Likewise if the morphine has stood for some minutes in the concentrated H_2SO_4 solution before adding the arsenate, this blue color is likely to be missed. Stand the test tube in a suitable bath (say of phos-

phoric acid), and heat. (This is much better than trying to heat it gradually and steadily over a free flame.) On heating to 30 to 40°C., or on standing for some minutes at room temperature, the color changes to green (or, if there has been no blue, a green color now develops). On continuing the heating, the color soon becomes dark green; at 60 to 70° it begins to change to blue, and soon becomes dark blue; at about 115° it changes back to dark green. This color, or green-black, persists until about 200°, when charring makes the solution a dirty brown-black.

Second Arsenate Test: Colors extracted by organic solvents after heating to 130°C.

Dissolve morphine in sulphuric acid, add arsenate, and heat, as described in the "First Arsenate Test," above. Heat to 120 to 145°C., preferably about 130°, then remove the test tube from the bath, cool, and dilute with about 6 c.c. water. A dark blue solution is formed from which a dark precipitate soon falls on standing. Divide into 3 or 4 portions, and shake each with an organic solvent. Amyl alcohol or chloroform extracts deep indigo or purple, ether crimson, and benzol deep violet-red. Nearly all the color is extracted from the aqueous layer in each case; ether, however, not being quite as good a solvent as the other three.

Third Arsenate Test: Colors extracted by organic solvents after heating to 175°C.

Heat morphine in sulphuric acid solution, with arsenate, as described above, but to 170 to 180°C., preferably 175°. (Use only 1 or 2 drops of the arsenate solution if the amount of morphine tested is quite small) Then cool and dilute with about 6 c.c. water. A blue-green-black solution is obtained, with a dark precipitate on standing. Amyl alcohol extracts deep (greenish) blue, chloroform green-blue, ether and benzol blue. Generally, considerable color remains in the aqueous layer, and chloroform or benzol may form an emulsion which prevents the color extracted from being seen readily. If a portion of the solution is made alkaline with concentrated NH_4OH, it turns a clear bright green, and from this solution amyl alcohol extracts green. These greens do not turn to brown as in the sulphuric-acid color test. In this "Third Arsenate Test," but not in the two preceding, heroine, codeine, and dionine all give the same results as morphine.

Dilution below 115°C.

If the morphine-arsenate solution is heated only to 105 to 110° (just before it turns from blue to green), and then diluted, it gives a green or olive-green solution in which a dark precipitate forms on standing, and from which solvents extract the same colors as after heating to 130°, but much weaker.

If the dilution is made after heating from 90 to 95° (when the concentrated acid solution is strongly dark blue), a yellowish olive-brown or olive-green solution is obtained, from which amyl alcohol will extract a little blue. Addition of ammonia to a portion of the aqueous solution makes it brownish red, and from this solution amyl alcohol extracts a red color.

If the dilution is made at 50 to 55° (when the concentrated acid solution is a strong green), a light yellow or orange-yellow solution is obtained, which cannot be depended on to yield a color to organic solvents.

Use of morphine hydrochloride:—This test has been restricted, in the description above, to the free alkaloid or the sulphate. The use of the hydrochloride of morphine tends to produce the effects of the halide-oxidation test. However, morphine hydrochloride may be used for the arsenate test as follows: Take no more than a moderate amount, dissolve in pure concentrated H_2SO_4, shake the tube and let stand a minute or so for the HCl gas to escape. Then add the arsenate. Color variable at first; blue, purple, red or brown, according to the extent of interference with the normal test. Heat, and the subsequent succession of colors should be as already described: at 30° becoming green, at 65° blue, at 115° dark green. On cooling and diluting after heating to 130° or 175°, the same results will be obtained as have already been described for the second and third arsenate tests. If dilution is made below 100°, it will be found that amyl alcohol will readily extract a blue-green color. Some other chloride effects may be found superimposed on the arsenate reaction, at these low temperatures.

Heroine.—Heroine gives the same reaction as morphine. Both for legitimate and illegitimate purposes it is commonly sold as the hydrochloride; therefore see the remarks above concerning the use of morphine hydrochloride for the arsenate test.

Codeine.—First Arsenate Test: Color changes in the sulphuric acid solution.

Codeine does not develop a color in the arsenate test so readily as morphine does. Unless the amount taken is rather large, there is no color until about 75 to 80°C. is reached; and in any case there is at first simply a gradually deepening purple color, and no actual change of color until about 115 to 120°C. is reached, when the solution becomes dark green, or green-black, as with morphine. The green color persists, as with morphine, until charring destroys it.

Second Arsenate Test: Colors extracted by organic solvents after heating to 130°C.

In this test the same colors are obtained with codeine as with morphine, but the solvents extract their colors much less successfully, leaving the aqueous layer decidedly blue. This is especially noticeable with ether, which extracts no more than a pink, leaving the aqueous layer dark blue, while with morphine, if shaken thoroughly, ether extracts practically all the color, becoming deep crimson.

Third Arsenate Test: Colors extracted by organic solvents after heating to 175°C.

In this test codeine reacts substantially the same as morphine.

Dilution below 115°C.

Diluted at 90°, when the concentrated acid solution is purple, codeine gives a solution but slightly yellowish, from which solvents do not extract any noticeable color. The similarity to morphine begins at about 100°, the results on diluting at 105°, as at 130°, being like those of morphine, but weaker.

Use of Codeine hydrochloride:—Fortunately the hydrochloride of codeine is a salt seldom met with. The presence of chloride will tend to cause the same reaction with codeine as with morphine, since the chloride-oxidation test is the same for the two alkaloids. However, if practically all of the HCl gas is allowed to escape before adding the arsenate, the color in the concentrated sulphuric acid solution (particularly at 50 to 60°, where morphine gives green) will be sufficiently different to distinguish the two alkaloids easily by means of this test.

Dionine.—The dionine reaction is naturally very like that of codeine, but there are differences, due to the fact that the ethyl-morphine compound breaks down more readily than the methyl-morphine compound. Dionine develops a purple color more readily than codeine, usually at about 50°C., or sooner. Dionine begins to react like morphine at about 75°, instead of 100° as for codeine. By the time dionine has been heated in concentrated sulphuric acid to 100°C., its reactions are practically identical with those of morphine after the same treatment; dionine, therefore, does not show those differences from the morphine reaction which distinguish codeine, after heating to 130°. As dionine is commonly sold in the form of the hydrochloride, caution must be used in distinguishing it by means of the arsenate test. See the remarks concerning the "Use of codeine hydrochloride," above.

COMPARISON OF THE SULPHURIC ACID COLOR TEST WITH THE THIRD ARSENATE TEST

	MORPHINE HEATED IN PURE CONC. H_2SO_4 TO 175 °C.	MORPHINE HEATED IN H_2SO_4 – ARSENATE SOL. TO 175°C.
Concentrated acid solution	Green-black	Green-black
Diluted with water	Dark blue-green	Dark blue-green
Amyl alcohol extracts	Deep blue (purplish)	Deep blue (greenish)
Ether extracts	No color, or faint blue	Good blue
Chloroform extracts	Scarcely any color, slight green	Deep blue-green
Ammoniacal solution	Clear green, changing on standing to olive, then brown	Clear green
Amyl alcohol extracts from NH_4OH sol.	Bright green (from green sol.); on standing overnight both layers brown	Bright green; on standing overnight both layers still green

III-A. THE CHROMATE TEST

"Potassium dichromate (added to the solution of solid morphine in cold concentrated sulphuric acid) is reduced with the production of a green color"—Allen's Commercial Organic Analysis.

This is a very misleading statement, as the color is due to the oxidized morphine, rather than to the reduced chromium. In fact, chromate is only a little less effective than arsenate in producing various colored compounds from morphine. There is this important practical difference, however, that chromate is so much more active an oxidizing agent that it must not be present in excess, otherwise the colored oxidation products will be destroyed on heating. Usually, therefore, the maximum effect of the total amount of morphine taken cannot be obtained; and so the chromate test, though of considerable interest, has no very great practical value.

Morphine, heroine, codeine, and dionine give substantially identical effects in the chromate test.

First Chromate Test.—If a little morphine is dissolved in concentrated sulphuric acid on a spot plate, and a small crystal of dichromate crushed and stirred in (as for the "Fading Purple Test" for strychnine), the solution becomes first brown, and then gradually brownish-green. If this solution is poured into a test tube, and diluted with about 2 c.c. of water, it gives an orange or reddish-orange solution, and on shaking with a little amyl alcohol the latter becomes bright green or bluish green. Thus morphine may be detected by means of the test for strychnine, but in testing for morphine specifically it is probably better to use the "Second Chromate Test" or to proceed as follows for the "First Chromate Test":

Put a little morphine in the form of the free alkaloid or the sulphate, in a clean dry test tube, and add (say) 8 drops pure concentrated H_2SO_4, or enough to dissolve the morphine readily. When it is dissolved, add 4 to 6 drops H_2SO_4 containing 0.2 gm. $Na_2Cr_2O_7$ per 100 c.c. This addition must be made in the cold, even though the solution is heated immediately after. In a very warm room it will be necessary to cool the acids beforehand. Shake the tube slightly to mix the solutions, then warm gently to 45° to 50°C., not above 60°. The reaction takes place rather gradually at room temperature; warming ensures oxidation as complete as the small amount of chromate permits. For mere traces of morphine the amount of chromate must be reduced. With a speck of morphine dissolved in 4 or 5 drops of H_2SO_4, and with the addition of but 1 drop of H_2SO_4 containing 0.1 gm. $Na_2Cr_2O_7$, I have obtained the reaction faintly. At low temperatures, even at ordinary room temperature, 20°C., there is little danger of the reaction being spoiled by too much chromate, but if any heat is applied, the chromate must not be in excess.

When the chromate solution is added and mixed in, the morphine solution becomes dark brown. At 45 to 50° it is dark greenish-brown or brownish-green. Dilute with a few c.c. of water. An orange or reddish-orange solution is obtained, from which amyl alcohol extracts a bright green or bluish-green color. Chloroform, benzol, and ether extract no color. Addition of ammonium acetate to a portion of the diluted solution changes its color to green; from this solution amyl alcohol extracts green. Na_2CO_3 added to the diluted solution changes the color first to green, then, with an excess, to brownish pink. Amyl alcohol extracts from this basic solution a greenish-blue to green color, leaving the aqueous layer salmon pink. This color remaining in the aqueous layer may be due to a trace of iron in the reagent.

Codeine cannot be distinguished from morphine in this reaction; it gives dark green rather than green-brown in the H_2SO_4 solution, but this is the only difference. Heroine and dionine, of course, react like morphine and codeine.

If the hydrochloride of the alkaloid is used, the previously-mentioned effects will be but little changed, but chloroform, ether, and benzol will extract colors. See the "Halide-Oxidation Test."

Second Chromate Test.—In the "First Chromate Test" a colored compound is formed which is soluble in amyl alcohol, but not in chloroform, benzol, or ether. If, however, the heating of the concentrated acid solution is carried above 70°C., these other solvents will also extract colors. As the effects are much the same over a considerable temperature range above this transition point, the simplest method of heating is to immerse the test tube in the boiling water-bath for some thirty seconds, or longer, even up to several minutes.

Put a little dry morphine (free alkaloid or sulphate) in a clean dry test tube, dissolve in a few drops pure concentrated H_2SO_4, add 4 to 6 drops H_2SO_4 containing 0.2 gm. $Na_2Cr_2O_7$ per 100 c.c., and stand the tube in the boiling water-bath for forty seconds, or longer. The concentrated-acid solution is then dark greenish-brown. Remove the tube, cool, and dilute with several c.c. water. A reddish or brownish-orange solution is obtained. A portion of this solution shaken with a little amyl alcohol yields a blue-green or green-blue color to the solvent. Chloroform extracts a deep purple or indigo, benzol pink, ether very slight pink. If a considerable quantity of ammonium acetate is added to the aqueous solution it becomes green. Amyl alcohol then extracts a dark blue, changing to dark blue-green; chloroform a deep purple or indigo (as before); benzol a pink color (stronger than before); ether a good magenta pink. Thus, the colors extracted by solvents are scarcely changed by the addition of NH_4Ac, but in some cases they are greatly strengthened. To extract any marked color with ether the addition of NH_4Ac is necessary; at the other extreme, chloroform extracts about as deep a color from the solution containing highly ionized sulphuric acid as from the one containing practically unionized acetic acid.

The Sulphuric Acid Color Test and the Chromate Test.—Since the morphine has to be in excess of the chromate, the excess morphine naturally reacts with the sulphuric acid, when a temperature of 140° to 150°C. is reached, as in the sulphuric-acid color test. But how does the morphine react which has already been oxidized by the chromate? We find that it reacts in the same way; that is, previous oxidation by chromate instead of spoiling the "Sulphuric Acid Color Test" strengthens it, with the addition of a few minor effects. The "third" and "fourth" chromate tests, described below, show the same colors as the "Sulphuric Acid Color Test," but stronger, and with the added effect of noticeable colors in other solvents than amyl alcohol.

Third Chromate Test.—Treat a little morphine with H_2SO_4 and chromate as before, this time heating to 155° to 160°C. The solution is dark brown. Cool and dilute with several c.c. of water. The aqueous solution is blue. Amyl alcohol extracts a deep violet color. Other solvents do not extract nearly so much color, but benzol extracts a readily noticeable lavender, ether a light pink, and chloroform a slight blue or purple.

Fourth Chromate Test.—Treat a little morphine with H_2SO_4 and chromate as before, this time heating to 175°C. The solution is green-black. Dilute with several c.c. of water. This gives a dark blue color. Amyl alcohol extracts purplish blue. Other solvents extract but little color; benzol a light violet, ether a light pink; chloroform a slight blue. Addition of NH_4OH to the dilute H_2SO_4 solution makes it clear yellowish green, and from this solution amyl alcohol extracts a green color.

III-B. THE PERCHLORATE TEST

"If the sulphuric acid solution (of morphine) be heated on the water-bath to 100° and a minute fragment of pure potassium perchlorate be added, a deep brown or reddish-brown coloration is produced, which rapidly spreads through the liquid. The color is destroyed on dilution. L. Siebold, to whom the test is due, did not observe a similar reaction with any other alkaloid."—Allen's Commercial Organic Analysis. The U. S. Dispensatory and Blyth (Poisons, Their Effects and Detection) also give this test. Blyth and Allen state: "The perchlorate must be free from chlorate which is ensured by heat-

ing it with hydrochloric acid as long as chlorine [ClO_2] is evolved. The salt is then washed with cold water and dried."

Blyth gives the reference: Am. Jour. Pharm., 1873. Apparently the test has not been in actual use since that time. Perchlorate intended for use in modern laboratories does not need purification in the manner stated. I have tried the test both with such perchlorate, and also with that precipitated by a potassium salt from perchloric acid solution. The latter being in very small crystals dissolves more readily in the concentrated sulphuric acid, and is more convenient to use. The test actually obtained is very similar to the arsenate test.

First Perchlorate Test.—Put a little morphine or its salt in a clean dry test tube, and dissolve it in a few drops of pure concentrated sulphuric acid. Add a very little solid potassium perchlorate, or better, 4 or 5 drops of a solution containing about 1 gm. $KClO_4$ in 100 c.c. pure sulphuric acid, for it is possible to destroy the reaction by too much perchlorate, and this must be guarded against if the amount of morphine is small. Stand the test tube in a suitable bath, provided with a thermometer, and heat. At about 60°C. a dark violet color develops. Should even a slight trace of chlorate be present the solution will, of course, become colored below 60°C., probably at room temperature: the perchlorate must be entirely free from chlorate. Continue the heating: the solution gradually becomes dark violaceous or brownish red rather than dark violet, then at about 125°C. it rapidly changes to dark green. Heat to about 150°, then remove the tube from the bath, cool, and dilute with about 1 c.c. of water for every two drops of H_2SO_4 used. The aqueous solution is a deep strong blue. Amyl alcohol readily extracts all the color, becoming deep violet or indigo. Ether and benzol are comparitively poor solvents, but extract readily noticeable pink and lavender-pink colors respectively. Chloroform is a poor solvent for the color, extracting only a slight purplish.

Second Perchlorate Test.—Proceed as in the "First Perchlorate Test," but reducing the amount of perchlorate somewhat, and carry the heating to nearly 175°C. The concentrated acid solution is still dark green. Dilution gives a very dark blue-green with a dark precipitate soon forming. Amyl alcohol extracts deep blue or indigo, ether a good violet or indigo color, chloroform light blue (greenish), and benzol a slight purplish-blue. Addition of ammonia changes the aqueous solution to green, and amyl alcohol then extracts green.

If the dark violet sulphuric acid solution is diluted without having been heated over 100°C., an orange-yellow solution is obtained from which amyl alcohol will extract only a slight blue. If the heating has been carried above 105°C., but not so high as to make the concentrated acid solution green, or if heating in the boiling water-bath has been continued for some twenty minutes, dilution gives an olive to dark green color, and organic solvents extract the same colors as when the heating has been carried to 150°C., but weaker. If, after heating to a little over 100°C., ammonia is added to the diluted solution it becomes salmon-pink, and amyl alcohol then extracts a pink color, leaving the aqueous layer light orange.

Heroine, codeine, and dionine give reactions in this test so much like that of morphine that they are not distinguishable. Unaccountably, codeine seems to give the test more readily than morphine.

While the perchlorate test is less complex, less distinctive, and less interesting than the arsenate test, it has one very considerable advantage over the latter: the reaction is not noticeably affected by the presence of chloride. The hydrochloride of the alkaloid, therefore, may be used as well as the sulphate or the free alkaloid. With bromide present, however, the "Halide-Oxidation Test" is obtained.

The perchlorate test should prove as useful as any, when one of this type is wanted.

IV. THE HALIDE-OXIDATION TEST

Several references have already been made to the fact that the presence of chloride or bromide greatly changes the "Arsenate Test" or the "Chromate Test." The purposive addition of sodium chloride, before oxidation by arsenate or chromate, provides an excellent test for morphine. Sodium bromide does even better, and can be used without the addition of an oxidizing agent, as hot sulphuric acid itself frees the halogen, in this case. Chloride, either with arsenate or chromate, and bromide, with or without the addition of arsenate or chromate, give results in the various combinations so similar that we certainly have to deal with only a single test, or reaction. For want of a better name, and to avoid any assumption, in naming the test, as to the nature of the reaction, I have called it the "Halide-Oxidation Test."

Bromide.—Put a little morphine or its salt in a clean dry test tube and add a little NaBr. Add 8 or 10 drops concentrated H_2SO_4 and shake the tube slightly to ensure contact of the acid with the morphine. Stand the tube in the boiling water-bath for about a minute. The colors obtained with solvents are practically the same regardless of the temperature used, so this is the simplest method of heating. The color of the concentrated acid solution varies from dark green, due to the morphine, to red-brown, due to the bromide. When taking the tube out of the water-bath, see that no morphine is floating undissolved on the acid. Dilution gives dark brown, soon dark green. Dilution to the extent of about one-half c.c. of water for each drop of concentrated H_2SO_4 gives a solution with a strong bluish-green color, in which a dark green precipitate soon forms. Amyl alcohol extracts deep green, benzol deep blue (or indigo). Chloroform on shaking well is colored at least green, with more morphine blue-green to deep blue; with much free bromine in the solution at first yellowish. Ether, shaken with the solution which is still warm from the dilution, readily extracts a violet or violet-blue color, but as the solvent cools (which, of course, occurs quickly) it deposits a green sediment on the sides of the test tube, losing most of its color, and thus appearing to change to a weak gray. These colors are but little affected by addition of NH_4Ac, $NaHCO_3$, Na_2CO_3, or NH_4OH.

The addition of an oxidizing solution of arsenate or chromate does not change the reaction, but assists in freeing bromine, to which the test seems to be due. By this means the test can be carried out very easily at room temperature; but if the solution is heated as described, the addition of an oxidizing agent is unnecessary.

Chloride and Chromate.—To a little dry morphine or its salt in a test tube add a pinch of NaCl, then 10 or 12 drops of concentrated H_2SO_4, shake the tube lightly, then at once add 5 or 6 drops of an H_2SO_4 solution containing 1 gm. $Na_2Cr_2O_7$ per 100 c.c. The concentrated acid solution is first dark brown, after warming (as described for bromide) dark greenish-brown. Diluted slightly with water the color is red; with further dilution the solution is clear light greenish-yellow to light yellow-green. Amyl alcohol extracts bright (bluish) green, chloroform greenish-blue, ether good violet, and benzol lavender or violet.

Note that much more chromate is used than can be used in the chromate test. The chloride takes care of the excess. Of course the chromate must not be in excess of both the morphine and the chloride together. Furthermore, it should not be added warm; on very hot days the acids should be cooled beforehand. Chloride and chromate give the least satisfactory form of the test.

Chloride and Arsenate.—Proceed as for chloride and chromate, but for the solution of the latter substitute 4 or 5 drops H_2SO_4 containing 5 gm. KH_2AsO_4 per 100 c.c. A red-brown color develops, on warming dark-brown or green-brown. Dilution with a little water gives deep red, further dilution oliveaceous-green. From this latter solution amyl alcohol extracts a deep blue or blue-green color, often appearing blue on looking directly through the solution, but blue-green when shaken up on the sides of the test tube. A yellow color is left in the aqueous layer. Chloroform extracts deep blue, benzol indigo or violet, ether reddish-violet. Reducing the acidity of the aqueous solution, even to making it slightly

alkaline, changes these colors very little, but greatly reduces the amount of color extracted by benzol or ether. Addition of NH_4Ac to the aqueous solution makes it green. Amyl alcohol then extracts deep green, chloroform good greenish-blue. $Na2CO_3$ or $NaHCO_3$ in excess makes the aqueous solution brownish-pink to pinkish-brown. Amyl alcohol then extracts a deep blue color, leaving the aqueuos layer slightly pink.

V. MISCELLANEOUS REACTIONS

There still remain undescribed in these articles some five tests or alleged tests for morphine or codeine which include heating in concentrated sulphuric acid solution. At the present time I am not considering any of the numerous tests which are carried out with cold concentrated sulphuric acid reagents: Froehde's reagent, Marquis' reagent, etc. The tests remaining are the phosphate, benzidine, arsenite, ferric chloride, and persulphate tests. They will now be described and discussed very briefly.

"*Phosphate Test.*"—"If sodium phosphate is substituted for arsenate and heat applied until acid fumes appear, the mixture becomes violet changing to brown or olive-green. If after cooling, water be gradually added, a reddish-brown coloration appears, changing to dirty bluish-green on further dilution. On shaking with chloroform the latter is colored blue."—Allen's Commercial Organic Analysis. This supposed test is also given by the U. S. Dispensatory, which credits it to Vulpius.

So far as I have been able to discover, the addition of phosphate (NaH_2PO_4) does not affect the sulphuric acid color test in the least, nor does it introduce any new effects.

It might be mentioned, however, that if the heating is carried out in an evaporating dish, instead of in a test tube, slightly different effects may be obtained, doubtless because in the evaporating dish the solution is more exposed to the air, which is capable of partially oxidizing the morphine-derivative in the hot sulphuric acid solution.

"*Benzidine Test.*"—"On heating 0.01 to 0.02 gm. morphine hydrochloride in a few c.c. concentrated H_2SO_4 containing 0.01 gm. benzidine, the liquid is first yellow, changing to brown, greenish-brown, and finally dark green. On adding this solution drop by drop to H_2O, a bluish-violet color is obtained. On shaking with $CHCl_3$, the latter extracts bluish, with the aqueous layer rose-red. NH_4OH added to the violet solution yields blue changing to green then greenish-gray. Codeine, dionine, heroine, apomorphine are similar," etc.—Chem. Abst., 1926, xx, 3330, credited to L. Ekkert: Pharm. Zentralhalle, 1926, lxvii, 498.

I have not been able to obtain any effect with benzidine. Very likely there has been a mistranslation, but in any case the description is suspiciously like that for the sulphuric acid color test.

"*Arsenite Test.*"—"Reichard states that if morphine is warmed gently with concentrated sulphuric acid containing arsenous or arsenic acid, an intense and permanent purple color is produced. The reaction is best obtained by dissolving the arsenous acid in a little strong solution of sodium hydroxide,

then add the morphine and finally an excess of concentrated acid.''—Allen's Commercial Organic Analysis. Blyth (Poisons, Their Effects and Detection) also gives this test.

It is certain that no intense and permanent purple color is obtained as stated.

Arsenous acid, As_2O_3, sometimes yields a pink or red color in such a test, more often, with morphine, no color at all. (At one time I thought this a test for codeine). I have not yet discovered just what are the necessary conditions for producing a pink or red color in this test.

Arsenic acid, As_2O_5, of course gives the arsenate test, but is not very convenient to use, as it does not dissolve readily in the sulphuric acid.

Confusion of As_2O_3 with As_2O_5 is not uncommon. An example is in Autenrieth-Warren (Detection of Poisons), where Gugliamelli's Reagents are given with arsenic trioxide, As_2O_3. I have tried, naturally without success, to obtain the reaction claimed for apomorphine with Arseno-tungstic solution, when the reagent is made as directed. The real test is simply the oxidation test for apomorphine, combined with a deep blue color in the aqueous layer due to the reduction of the Arseni-tungstic solution, made with As_2O_5. The same remarks doubtless apply to the As_2O_3 of the Arseno-tungsto-molybdic solution, though I have not tried it. The mistakes possibly arise through mistranslation.

Ferric chloride test for Codeine.—Warmed with sulphuric acid containing a little ferric chloride, codeine gives a deep blue color. This is one of the tests for identity of codeine in the U. S. Pharmacopoeia. With only a little alkaloid, heating to as much as 80°C. may be necessary. The color will develop only very slowly in the cold. A convenient way of obtaining the reaction in ordinary cases is to dilute a drop or two of $FeCl_3$ solution with H_2SO_4 to a light yellow color and apply this reagent while still warm (from the dilution) to the codeine on a spot plate. The blue color then develops at once, if a fair amount of codeine is used. Morphine in such a test gives no color. Heated to 80°C. in H_2SO_4 containing $FeCl_3$ morphine gives a comparatively weak, dirty violet color, or may even, under proper conditions, give blue.

Blyth says, ''This blue color is apparently common to all ethers of the codeine class.'' (Poisons, Their Effects and Detection). Even so, the test is a fairly good one for distinguishing codeine from morphine.

Persulphate test.—If morphine is dissolved in concentrated sulphuric acid and a little ammonium persulphate in sulphuric acid added, a yellow color is produced which changes to green. Heating produces an orange-brown color. Dilution of the green (or orange-brown) solution gives a yellow solution from which amyl alcohol extracts merely a yellow or orangish-yellow color. The reaction therefore has no qualitative interest aside from the green color developed in the concentrated acid. Codeine reacts like morphine.

VI. NATURE OF THE REACTIONS

Apomorphine and Dehydrated Morphine.—When morphine is heated in concentrated phosphoric acid, in a saturated solution of zinc chloride, or in concentrated hydrochloric acid under pressure (or in the presence of a drop or two of concentrated H_2SO_4), it is dehydrated, and apomorphine is formed. When apomorphine is oxidized it is converted into a compound which is bright green in aqueous (nearly neutral) solution, crimson to magenta-pink in ether, blue in amyl alcohol, etc. This is known as Pellagri's test for morphine.

When morphine is heated in pure concentrated sulphuric acid and the solution then cooled and treated with a tiny crystal of potassium nitrate or some similar oxidizing agent, a magnificent deep red color is obtained. This is known as Husemann's test. Most authorities intimate, and some of them say outright, that this reaction is due to apomorphine. It seems natural that dehydration by sulphuric acid should give the same compound as dehydration by phosphoric or hydrochloric acid. Nevertheless, this idea is entirely erroneous.

When morphine is dissolved in pure concentrated sulphuric acid and the solution warmed slightly, then diluted with water, oxidized with iodic acid, and neutralized, a bright green color is obtained, and amyl alcohol extracts blue. Thus far it seems as though apomorphine must have been formed, but ether, and also chloroform, benzol, etc., entirely fail to extract any color whatever. From the different solubilities of the oxidation product it is clear that the compound formed is not apomorphine.

A study of this "Derivative-Oxidation Test," as I have called it, shows that the change begins as soon as the morphine is dissolved in the acid, and takes place gradually at room temperature; and that the green color in aqueous solution is strengthened (when only a few minutes are allowed for the action of the acid) if the concentrated acid solution is heated at least to 35° C., and reaches a maximum at not over 50° C.

When morphine is heated in pure concentrated sulphuric acid to 40° to 50° C., and the diluted solution tested with such general alkaloidal reagents as phosphomolybdic acid, Wagner's reagent, Mayer's reagent, and gold chloride, no precipitate is obtained, though these reagents are all very sensitive to morphine and to apomorphine also. This shows again that the compound formed is not apomorphine, and it also shows that morphine is completely converted to an entirely different compound by the action of sulphuric acid, in a few minutes and with very little heating. It would seem that the compound formed is not even an alkaloid; but there are several recognized alkaloids which are not precipitated by most of the general alkaloidal reagents. When the diluted acid solution is allowed to stand, without oxidation or other treatment, the morphine derivative is precipitated, a colorless, crystalline compound. Its color reactions are similar to, some identical with, those of apomorphine, including a color reaction with ferric chloride. Like morphine and apomorphine, but unlike most alkaloids, it is readily soluble in bases.

The statements in the textbooks as to the compound formed on heating morphine in sulphuric acid seem to be nothing more than poor guesses. As already mentioned, several authorities say that apomorphine is formed, on heating at 100° C. Witthaus (Manual of Chemistry) says that di- and trimorphine are formed at that temperature. Prescott (Organic Analysis) says that on heating at 150° to 160° C. sulphmorphide is formed, a substance similar to apomorphine sulphate. As we have seen, a far less degree of heat than is usually given is sufficient to effect a complete change, and a change more radical than any of those indicated. It may be a change of polymerization or molecular rearrangement, or possibly even one of mild oxidation; but

since the color reactions of the compound formed are so similar to those of apomorphine, it seems most probable that this compound also is dehydrated-morphine. Since it is not identical with apomorphine, we must suppose that molecular rearrangement or polymerization takes place in addition to dehydration. One authority (I cannot locate the passage at the present time) suggests that apomorphine itself is polymerized. No doubt these problems can be solved easily by analysis, but thus far I have had time only for working out the qualitative tests.

Dehydrated Codeine and Apocodeine.—When codeine is warmed in pure concentrated sulphuric acid to 40° to 50° C., and the solution cooled and diluted, a crystalline compound is precipitated on standing, just as with morphine. Codeine gives the derivative-oxidation test the same as morphine. Now codeine itself differs from morphine, qualitatively, in two respects: it is not a reducing agent (in aqueous solution), and so does not respond, for instance, to the test with iodic acid and ammonia, and it has no phenolic hydroxyl group, and so gives no color with ferric chloride. The codeine derivative formed in sulphuric acid at 40° C. is a reducing agent, being readily oxidized, for instance, by iodic acid. But it is not a phenol, if we may judge by its failure to react with ferric chloride, whereas the morphine derivative shows the same phenolic color reactions as apomorphine.

Thus dehydrated codeine is quite distinct from dehydrated morphine, at least when the dehydration is accomplished by means of concentrated sulphuric acid. The existence of apocodeine as a definite compound has been called in question. "D. B. Dott doubts the existence of apocodeine, and states that commercial *apocodeine hydrochloride* is not of a very definite nature, being probably a mixture of an amorphous modification of codeine, polymerized bases, chlorocodide, and apomorphine."—Allen's Commercial Organic Analysis. It is probably significant that Stephenson, in his investigation of microchemic tests for alkaloids, was unable to obtain a single crystal with apocodeine, although it gave precipitates with all the common alkaloidal reagents. Certainly this is not suggestive of a pure substance.

All this may be true of commercial apocodeine; but since codeine retains one of the two characteristics by which it differs from morphine when dehydrated by sulphuric acid, it seems to me that it should retain the same characteristic difference when carefully dehydrated by phosphoric acid. Experiment bears this out, for when codeine is boiled just a moment in 85 per cent phosphoric acid it will give the oxidation test (Pellagri's), but not the iron test, whereas with morphine the two tests go together. To be sure, some authorities say that when dehydrated by concentrated hydrochloric acid codeine first forms chlorocodide, and then apomorphine. The whole subject is presented in a very confused and contradictory manner; I may at least succeed in getting some one who really knows to clear it up.

In *Chemical Abstracts* for 1926 (xx, 1469), I notice the following: "Apparently the experiments hitherto performed with apocodeine have been made with impure preparations. Crystalline apocodeine is some five times as active as the amorphous commercial apocodeine. On the other hand there is no im-

portant qualitative difference between the (pharmacologic) action of the pure and the commercial preparations, etc."—Otto Krayer, Arch. Exper. Path. Pharmacol., 1926, cxi, 60-7.

Changes on Further Heating, and in the Presence of Oxidizing Agents.—In Husemann's test the directions are, usually, that the morphine be dissolved in sulphuric acid and heated to, or at, 100° C. If heated only to 40° the reaction is not quite the same. In the "Derivative Oxidation Test" the morphine is heated in sulphuric acid to 40° C. If heated to 100° instead, the same green color is produced in the aqueous layer when the test is applied, but amyl alcohol will no longer extract any blue color whatever. A study of these and other reactions shows that when morphine (or codeine) is heated in pure concentrated sulphuric acid a change takes place at 60° to 70° C. It is a minor change, a slight rearrangement of the atoms in the molecule, or a change in polymerization. The derivative will no longer crystallize out of the diluted solution.

On continuing the heating to 140° and above, a dark green color develops. As dehydration has already taken place, presumably, we naturally think of decomposition or oxidation, or both. Hot concentrated sulphuric acid is an oxidizing agent, and at the temperature of the change it is very nearly fuming. Morphine itself is so readily oxidized as to be classed as a reducing agent; when dehydrated it is still more easily oxidized; it is, therefore, not surprising that hot sulphuric acid oxidizes it at 140°.

The sulphuric acid color test reaches its maximum about 175° C., with a slight change in the colors obtainable in organic solvents occurring at about 165°. Now when arsenate, chromate, or perchlorate is present, we have oxidation taking place even below 100°, but this does not prevent the formation of practically the same colors above 140°, as in the sulphuric acid color test. It seems, then, that on heating in pure sulphuric acid, oxidation takes place at 140° in much the same way as in the presence of chromate at the same temperature, or with perchlorate at 125°, or with arsenate at as low as 115°. The change at 165°, which takes place in all four cases, is then probably one of decomposition. It takes place subsequently to the oxidation when an oxidizing agent is present, but concurrently with the oxidation which has begun but which at 165° is still incomplete, in pure sulphuric acid.

Chromate is an energetic oxidizing agent, and can be present only in very small amount. Concurrent oxidation and dehydration are then produced. Arsenate is a milder but very effective oxidizing agent. That the action of the two is substantially the same is shown by the similar effects produced in the chromate test and the arsenate test, and further by the fact that in the presence of chloride or bromide the same reaction is obtained with both. Perchlorate is an oxidizing agent still milder (at low temperatures) than arsenate; with it dehydration takes place before oxidation, but in the same solution and in the same continuous operation. In the presence of arsenate or chromate the same kind of change takes place at 60° to 70° C. that we have already noted for pure sulphuric acid, and these temperatures also mark the beginning of the perchlorate test.

The weak blue color that is first obtained in the arsenate test, likewise the dark brown or red-brown first produced by chromate, seems to be due to the oxidation of the morphine as such. It does not interfere with the subsequent dehydration.

Sodium chloride when warmed with chromate in sulphuric acid is of course oxidized, chlorine being set free. Apparently it is the free chlorine or bromine which makes the halide-oxidation test distinctive and different from the arsenate or chromate test. No new effect could be found with sulphite added, in the chromate test. Thus in the presence of chloride, the oxidizing agent actually added operates only indirectly; and an indirect

THE CHANGES WITH TEMPERATURE ON HEATING MORPHINE IN CONCENTRATED SULPHURIC ACID*

	HEATING IN PURE H_2SO_4	ARSENATE TESTS	CHROMATE TEST PERCHLORATE TEST
15°–20° C.	Morphine gradually converted to comp. insol. in dil. acid, and with color reactions like Apomorph.	Morphine gives blue color in H_2SO_4–Ars. sol.	Chromate test: Amyl alc. ext. green from dil. sol., other solvents no color
30°–40° C.	Same change more quickly.	Morphine color becomes green in H_2SO_4–Ars. sol.	Chromate test: same reaction sooner complete
50° C.		Dionine gives purple color	
60°–70° C.	Compound becomes sol. in dil. acid, minor changes in color reactions occur	Morphine color becomes blue in H_2SO_4–Ars. sol.	Chromate test: $CHCl_3$, ether, benzol hereafter ext. colors from dil. sol. Perchlorate test: begins; deep violet in conc. acid sol. develops
75°–80° C.	Dionine-derivative begins to react like that of morph. to $FeCl_3$	Codeine gives purple color. Dionine begins to react like morphine	
100°	Codeine-derivative begins to react like that of morph. to $FeCl_3$	Morphine gives green on diln. and org. solvents ext. colors. Codeine begins to react like morphine	
105°			Perchlorate test: green on diln. and org. solv. ext. colors
115°		Color changes to green in H_2SO_4–Ars. sol; Organic solv. ext. strong colors	
125°			Perchlorate test: color changes to green in conc. acid sol; Amyl alc. ext. deep color and other solvents some
140°	H_2SO_4 sol. becomes dark olive-green; Amyl alc. ext. slight violet from dil. sol		Chromate test: colors on diln. and extn. with Amyl alc. practically as with heating in pure H_2SO_4; stronger
165°	Amyl alc. ext. blue from dil. sol., green from ammoniacal sol	Colors extracted by organic solvents change; in amyl alc. nearly same as with heating in pure H_2SO_4	Chromate test: colors change as with heating in pure H_2SO_4, nearly Perchlorate test: colors change as with heating in pure H_2SO_4, nearly
180°	Charring set in		

*Note: Temperatures are approximate, and show when changes have noticeably begun, not when the effects are strongest.

oxidation can be effected by concentrated sulphuric acid even at low temperatures, through the medium of sodium bromide.

It is just when one would least expect it, in sulphuric acid solution, that it makes a great difference whether the morphine is in the form of the hydrochloride or the sulphate.

SUMMARY

I. Sulphuric Acid Color Test. Morphine gives a color reaction on heating in pure concentrated sulphuric acid to 140° C. and above. For a qualitative test the heating is carried to 175°. The colors extracted by amyl alcohol from the diluted solution are especially useful. Codeine gives the same test.

II. Arsenate Test. Morphine gives three qualitative color tests with sulphuric acid containing a little arsenate, as follows:

1. A succession of colors in the concentrated acid solution on heating.
2. Distinctive colors extracted from the diluted solution by organic solvents, after heating to 130° C.
3. Distinctive colors extracted from diluted solution by organic solvents, after heating to 175° C.

The second is the best qualitative test, and an excellent one. Codeine does not give quite the same reaction as morphine. Although many authorities mention the arsenate test, existing descriptions in books are without statements of the requisite temperatures, and fail to recognize the interference of chloride or bromide.

III. Chromate Test and Perchlorate Test. Morphine gives a reaction with chromate similar to that with arsenate. Four phases are distinguished, providing separate tests: Colors extracted by solvents after heating to (1) 40°, (2) 100°, (3) 155°, (4) 175° C. The chromate must not be in excess of the morphine, and the test is not of much practical value.

Perchlorate gives a test similar to the arsenate test, but not so complex. No color develops until the concentrated acid solution has been heated to about 60° C. Two qualitative tests are distinguished, as follows:

1. Distinctive colors obtained by heating to about 150° C.
2. Distinctive colors obtained by heating to nearly 175° C.

The first of these tests is the better, and should be a useful test of this type. Chloride does not interfere with the perchlorate test.

IV. Halide-Oxidation Test. Morphine when heated with sodium bromide in concentrated sulphuric acid solution gives a colored product which organic solvents extract from the diluted solution with bright distinctive colors. Codeine gives the same test. This reaction appears to be due to the bromine set free by the concentrated sulphuric acid, for when chloride is substituted for bromide, and an oxidizing agent (arsenate or chromate) added practically the same reaction is obtained. This is a new and sensitive qualitative test. Because of this reaction, it is desirable and to some extent necessary

that morphine (or codeine, heroine, dionine) for the arsenate (or chromate) test should not be in the form of the hydrochloride.

V. Miscellaneous Reactions. A brief discussion is given in this article of the reactions or supposed reactions of morphine and codeine on heating with phosphate, benzidine, arsenite, ferric chloride, or persulphate, in sulphuric acid solution.

VI. Nature of the Reactions. In this article the relationship of the various tests is discussed together with the changes produced on heating morphine in pure sulphuric acid. An attempt is made to explain the changes taking place in these tests as the result of dehydration (although no apomorphine is formed), due to the concentrated sulphuric acid, with concurrent oxidation (in the reactions discussed in the present paper), due to the arsenate, chromate, or other oxidizing agent added.

General: As qualitative tests, the following are the best of those described:

1. The Halide-Oxidation Test, using bromide.
2. The Perchlorate Test, with heating to about 150° C.
3. The Arsenate Test, when the alkaloid is present as the sulphate or is free; with heating to about 135° C.

The interference of chloride should be considered a possibility, prior to investigation, in all tests in which morphine or one of its derivatives is oxidized in concentrated sulphuric acid solution. As heroine and dionine are usually, and morphine often sold in the form of the hydrochloride, this is an important matter.

It has become evident that each oxidizing agent has its characteristic reaction with morphine. Additional tests similar to those described in this paper will no doubt be discovered in the course of time, but it is hardly worth while to look for them. Plenty of good tests for morphine are now known; it is only necessary to separate the good from the bad. An authoritative general review of the tests for morphine should be made, and then analytic investigators should turn their attention to other alkaloids.

REFERENCES

[1]Allen's Commercial Organic Analysis, ed. 4, 1912.
[2]Blyth: Poisons, Their Effects and Detection, ed. 5, 1920.
[3]Autenrieth-Warren: Detection of Poisons, ed. 5, 1921.
[4]Fuller: Chemistry and Analysis of Drugs and Medicines, 1920.
[5]United States Dispensatory, ed. 20, 1918.
[6]United States Pharmacopoeia X, effective 1926.
[7]Fresenius-Wells: Qualitative Chemical Analysis, ed. 16, 1915.
[8]Prescott: Organic Analysis, 1887.
[9]Perkin: Qualitative Chemical Analysis, 1901.
[10]Peterson, Haines, and Webster: Legal Medicine and Toxicology, ii, ed. 2, 1923.
[11]Witthaus: Manual of Chemistry, 1902.
[12]Stephenson: Some Michrochemical Tests for Alkaloids, 1921.
[13]Levine and Magiera: Studies in Toxicologic Chemistry, II: The Formaldehyde Sulphuric-acid Reaction of the Opium Alkaloids. JOUR. LAB. AND CLIN. MED., May 1927, xii, 773.
[14]Fulton: Some New and Improved Tests for Morphine and Related Alkaloids. JOUR. LAB. AND CLIN. MED., May, 1928, xiii, 750.

DISEASES OF THE MOUTH*

By Sterling V. Mead, D.D.S., Washington, D. C.

HEALTH is our greatest asset. A clean mouth and sound teeth have a tremendously important bearing on the physical condition and efficiency of an individual. The mouth is subject to as great a variety of diseases as any other part of the system, although their multiplicity is not always appreciated. Diseases of the mouth may be either local in character or they may constitute part of the picture of general disease. While it has been recognized by many for years that oral sepsis causes many systemic disturbances, it has been only recently that we have learned to know the advantages and real necessity for a close cooperation between physicians and dentists in handling these cases. The dentist has learned that he may usually expect the most unselfish cooperation from the physician, and in return it is expected of him that he limit his activities where the service can be better performed by the physician, surgeon, or specialist, and endeavor to aid the medical man in his effort to eliminate all pathologic mouth conditions.

While it has been common practice in the past for the dentist to consider the maxillary sinus his special field, I am sure it has been our experience in Washington and in many other localities, that the most good may be accomplished by the dentist handling the mouth factors and lending any further service he can to the rhinologist. This is true of many other medical and surgical problems. I believe that the sooner mouth wounds communicating with the maxillary sinuses are closed, and further treatment turned over to the rhinologist, the better the results.

In the study of treatment of diseases of the mouth, I believe it is generally conceded that diagnosis is of paramount importance. A correct diagnosis is imperative before the prognosis can be given and the proper treatment instituted. There can be but one true diagnosis, but many forms of treatment in the hands of different men may accomplish the same result. A thorough understanding of all of the diagnostic methods is essential for the successful treatment of a patient who has come to the physician or to the dentist for the relief of disturbing symptoms.

A mouth examination should be conducted in a routine manner and the results recorded upon some suitable record chart.

A very common misconception of the proper diagnostic methods is the practice of many physicians in referring patients to the dentist for a radiographic survey of the mouth with a request for a radiographic report, and there is a very prevalent idea that the only prerequisite for a mouth diagnosis is the ability to take good radiograms. If a radiographic examination is to

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be of any great value, it should be taken by a dentist or physician schooled in x-ray technology and the fundamental principles of diagnosis, and whose experience in this field gives a more correct interpretation. The fact should not be overlooked that radiograms of the teeth are in most cases useless without considering the findings of the other diagnostic methods such as history, manifestations of pain, general oral examination, percussion and palpation, exploration, color, conductivity of temperature, transillumination, electric pulp test, bacteriologic examination, histopathologic examination, blood, and other laboratory methods. There also seems to be a very general idea that the pulpless tooth is the only source of oral sepsis, causing systemic disturbances. This is only one of many factors. A vital tooth may in some instances show periapical disease. Periodontal diseases, and especially periodontoclasia, are present in the mouths of most adults.

Other etiologic factors to be considered are: stomatitis of various types, impacted and unerupted teeth, fractures, periostitis, rarefying and condensing ostitis, acute and chronic osteomyelitis, necrosis, ostitis serrata, exostosis, myositis ossificans, radicular, follicular and multilocular cysts, and benign and malignant tumors, etc.

The value of radiography should not be misunderstood. It is relative, as one views the abnormal area by contrast. A radiolucent or radiopaque area may be hidden or distorted by exposure from different angles, interposing tooth roots or dense bone, and thus many infected areas may be overlooked. Pathologic areas and areas undergoing regeneration following operation should be compared with other similar areas in the mouth and contrasted. Good radiograms are rarely misleading if properly interpreted, but their possibilities are limited and the vital factor in determining the presence or absence of disease must be the final clinical examination. It has been my experience that the postoperative results are usually more pronounced than the radiogram would indicate.

Inflammation is a cellular defensive reaction against injury, and infection is not the only element causing this injury, as it may be due to trauma, chemicals, heat, cold, or electricity.

As a result of inflammation rarefaction or condensation of bone may result, or they may both occur simultaneously. Therefore, rarefaction is indicative of inflammation, which may possibly be from infection, and causes the radiolucent radiographic appearance. Inflammation may cause an increased density of the bone lamella and results in a radiographic radiopacity.

The electric pulp tester is a valuable diagnostic agent when used with transillumination and the radiogram, but is not reliable when used independently.

Transillumination of the teeth, gums, and sinuses is indispensable. The radiogram may be absolutely negative as there may be no bone pathology shown and transillumination may show definite disease, and in some cases both of these methods may be negative and disease may be present. Transillumination is a valuable aid in making a diagnosis of mouth conditions, but it has its limitations. It is unreliable in disclosing areas of periapical infection, and it does not disclose bone pathology, except where it extends to the

soft tissues. In other words, the pathology, shown by transillumination, is due to soft tissue infection.

The fact that the alveolus and gums do not show a shadow, when transilluminated, does not eliminate the possibility of infection. My experience is that it is usually not possible to detect periapical infection by transillumination where the periapical disease is not accompanied by periodontal disturbances.

One of the perplexing problems of oral diagnosis is the unerupted or impacted tooth, as it should always be considered questionable even though no definite symptom of pathology is shown. While it is possible in some instances for pressure of an impaction to give reflex disturbances, I do not believe this usually occurs without local symptoms, but I do believe that the ostitis and infection about the impaction are the important etiologic factors so far as systemic disturbances are concerned.

The removal of impacted third molars more than any dental operation is attended with serious consequences. Anyone who has attempted much of this work will have experienced some very serious results. While, as many of you realize, this in many cases is a dangerous procedure, it is difficult to educate the public that it is as important to have the proper postoperative care in these cases as in tonsil and other operations.

It takes experience to teach one that it is better not to remove one of these teeth, than to do so and have the patient not remain or fail to return for treatment. I believe we will learn to hospitalize these patients more and more.

A periodontal disease is a disturbance of the periodontal tissues. It includes all diseases of the tissues surrounding the tooth root which have their origin in the gingivae, periodontal membrane, and gums.

The term "pyorrhea" which is quite widely used in speaking of these conditions has given rise to a great deal of confusion, as pyorrhea, or Rigg's disease, is described as a loosening of the teeth associated with continued suppuration. There are various stages of periodontal involvement, and the initial lesion is not associated with suppuration, but only with simple inflammation. The term "pyorrhea," therefore, does not convey any idea as to the state of involvement of the tissues. Under periodontal disease we consider the different forms of recession, gingivitis, hypertrophy, pericementitis, and periodontoclasia.

Unfortunately the pulpless tooth problem is one of the phases of our work upon which there is a difference of opinion.

Radiographically the pulpless tooth may be functional and harmless if the lamina dura is definite, if the surrounding bone cancellations appear to be normal, as compared with adjacent normal teeth; if there are no clinical symptoms of disturbance; if the apex is not hypoplastic and there is a filling well into, but not through, the apex.

In doubtful cases where the vitality test, transillumination, radiograms, and other methods do not show definite infection and it is suspected, the health of the individual must be taken into consideration. The pulpless tooth is in the same category as the tonsils. The diagnosis cannot always

be made from the local appearance, but general symptoms must be considered. It is not always possible to examine a tonsil or a pulpless tooth and give a positive assurance that there is no pathology, even in the absence of any apparent infection. Where sepsis is known to exist, and other foci have been eliminated or ruled out, it may be necessary to remove pulpless teeth or to open into suspected pathologic bone areas throughout the mouth, even though no definite infection is apparent.

I do not agree with the radical who condemns every pulpless tooth. I am not pleading for the pulpless tooth, for I am very much afraid of it; but I do believe if the patient will present himself in time, and the pulp is removed aseptically and the canals filled properly that a large percentage of such teeth may be retained. I do not believe an infected tooth apex should be retained a minute, and I would consider it a waste of time to attempt to treat one unless an apiectomy is performed in a selected case.

In determining when to extract a pulpless tooth, it is necessary to take into consideration many factors. Each case should be handled as good judgment and experience indicate, and with but very few exceptions, my practice is to recommend extraction of:

1. All teeth showing evidence of periapical bone involvement.

2. All pulpless teeth in patients whose health is seriously endangered.

3. All pulpless teeth showing marked periodontal bone resorption (periodontoclasia).

4. All pulpless teeth directly bordering on the maxillary sinus.

5. All third molars when pulpless or causing pressure or decay in other teeth.

6. All pulpless teeth when the root formation precludes the possibility of a good root filling.

7. All pulpless deciduous teeth.

If I were to make any specific recommendation for proper cooperation of physician and dentist, I would call attention to the advisability of personal contact. The patient cannot be trusted with reports, as they are so apt to mistinterpret them. If the physician refers a case to the dentist, it would be well to telephone or write to him giving him some idea of the patient's general condition, and what is to be determined. In many cases, it is necessary for the dentist to see the patient after radiograms and other evidence are completed so that a proper diagnosis may be made and an intelligent report and recommendation made. Patients should be taught the value of preoperative and postoperative radiographic examination, as well as laboratory and other diagnostic methods.

It is not enough that a physician advise a patient to go to his dentist and have his teeth looked over. In many instances the patient is at fault when the physician receives an unsatisfactory report, as he fails to advise the dentist what is wanted.

It is inconceivable and unfortunate that after more than fifteen years of close study on the part of medicine and dentistry, there is still a divided

opinion regarding the rôle of oral sepsis. Dentistry has many unsolved problems and much should be accomplished in the field of prevention, and care of the mouth.

We hear a great deal about education of the public in regard to early recognition of stomatitis and tumors of the mouth, but I wonder just how many physicians and dentists are able to diagnose these mouth conditions when they see them. I believe that it is apparent to all that there is great need for a more thorough study of mouth conditions, and proper training of both the physician and the dentist, to enable him to recognize the character and significance of the many oral abnormalities.

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PARATHYROID EXTRACT AND GLUCOSE TOLERANCE IN DIABETES MELLITUS*

By Max Wishnofsky, M.D., and Jesse M. Frankel, M.D., Brooklyn, N. Y.

IN A PREVIOUS paper one of us[1] discussed the influence of calcium on carbohydrate metabolism. It was shown that there occur simultaneously changes in calcium and carbohydrate metabolism in disturbances of the adrenal, thyroid, parathyroid, and pituitary glands and in various miscellaneous pathologic states. The latter half of the paper was devoted to the discussion of the relationship of calcium to carbohydrate metabolism in diabetes mellitus. It will be well to review some of the statements made.

Von Moraczewski[2] found an inordinate excretion of calcium in one case of diabetes mellitus. A year later he reported cases where the excretion of sugar was reduced by the daily ingestion of ten grams of calcium phosphate.[3] Von Noorden[4] observed uniformly a negative calcium balance in diabetes. This was out of proportion to an increased calcium elimination that might be caused by an existing acidosis. Falta and Whitney[5] found that pancreatectomy was associated with an immediate and rapid elimination of calcium. This was far in excess of that which could be accounted for by the mild acidosis present. The most elaborate work on the relationship between calcium and diabetes mellitus was performed by Kahn and Kahn.[6] They first showed that out of five cases examined all showed a definite negative calcium balance. The patients were kept on a standard diet for three days during which time the glycosuria and glycemia were determined. On the same diet they were then injected intravenously with varying amounts of eighth molecular calcium chloride every few days. The glycosuria and glycemia were determined to observe the effects of the treatment. As a result of these experiments they concluded that calcium administration definitely reduces glycemia and glycosuria in diabetes. More recently the following work has been done. Davies,

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Dickens, and Dodds[7] observed that in rabbits the injection of insulin produced a decided increase in blood calcium. Rothchild and Jacobsohn[8] observed the same finding in man; this could not be confirmed by Harrop and Benedict.[9]

Employing the glucose tolerance test as an index of the severity of diabetes, it was shown by one of us[1] that calcium administered intravenously in no way ameliorates the diabetic state.

It was decided to continue this work and study the influence of parathyroid extract on glucose tolerance in diabetes mellitus. This subject is interesting from two standpoints: first, the parathyroids are of the glands of internal secretion and many of the latter are intimately associated with carbohydrate metabolism; and secondly, the parathyroids exert a potent influence on calcium metabolism or to put it in the words of Collip:[10] "The function of the parathyroid hormone appears to be that of a regulator of calcium metabolism, and its action is primarily as a calcium mobilizer."

Eppinger, Falta, and Rudinger,[11] also Marine,[11] observed a diminished tolerance for glucose in parathyroidectomized dogs but not in the tetany of man. Underhill and Blatherwick[12] observed that thyroparathyroidectomy causes hypoglycemia, and that the normal glucose level is restored by calcium injections. They concluded that calcium may play an important rôle in maintaining the equilibrium of the blood-sugar regulating mechanism.

In discussing carbohydrate metabolism two important phases must be considered: one, the oxidation of glucose, which can be studied from the respiratory quotient; and two, the storage of carbohydrates (glycogenesis and glycogenolysis). These two phases are separate and distinct. Adrenalin engenders an increase in the oxidation of glucose and glycogenolysis. Insulin favors glycogenesis and an increase in the oxidation of glucose. Cammidge and Howard have made some interesting studies on the influence of the various hormones on carbohydrate metabolism. Only those findings that are relevant will be mentioned. It was found[13] that the injection of parathyroid extract into fasting rats and guinea pigs caused no change in the respiratory quotient. The injection of parathyroid extract and one-half unit of insulin, however, produced a change in respiratory quotient and oxygen consumption resembling that previously found to follow the injection of two units of insulin alone. They studied also[14] the influence of the various hormones on the glycogenolytic activity of diastase in vitro. Adrenalin accelerates glycogenolysis. Thyroid extract has no influence on glycogenolysis, but it markedly augments the action of adrenalin. Insulin diminishes glycogenolysis. Parathyroid extract by itself has no influence on glycogenolysis, but it increases considerably the inhibiting action of insulin. They conclude as follows: "It would seem that the parathyroids are related to the internal secretion of the pancreas in much the same way as the thyroid is to the internal secretion of the adrenals."

Winter and Smith[15] observed that parathyroid extract alone had no effect on blood-sugar concentration; but when combined with insulin only one-third to one-fourth the usual dose of the latter was necessary to induce convulsions. Forrest[16] also noticed that parathyroid extract had no influence on the blood-

sugar level; but that when it was combined with insulin it caused a more marked lowering of the blood-sugar concentration than when insulin was given alone. Petty, Stoner, and Schaffer[17] did not find it possible to reduce the insulin dosage in severe diabetics by using parathyroid extract.

It may be stated that the administration of glucose causes an outpouring of insulin into the circulation of all individuals except absolute diabetics. The amount of insulin discharged depends on whether the individual is normal or diabetic, and in the latter on the severity of the diabetes. If we are to accept the findings of Cammidge and Howard,[13, 14] namely, that parathyroid extract augments the capacity of insulin for glycogenesis and the oxidation of glucose, then the carbohydrate tolerance of a diabetic to whom glucose and parathyroid extract have been given should be increased. This paper is devoted to the study of the influence of parathyroid extract on the glucose tolerance test in diabetes mellitus.

A direct and accurate method of estimating the tolerance of an individual for carbohydrates is the glucose tolerance test. A normal individual when given 1.75 grams of glucose per kilogram of body weight on a fasting stomach shows a blood-sugar curve as follows: the maximum increase usually occurs at the end of one-half hour and is never more than from 30 to 50 per cent above the fasting level; at the end of two hours the fasting level should be regained. No glycosuria should occur. In diabetics the curve is equally characteristic; the maximum increase is much greater and the fasting level is not regained for many hours. Glycosuria occurs depending on the height and length of the curve and the renal threshold of that individual for glucose. The indices of the severity of any case therefore are: the height and length of the blood-sugar curve and the amount of glycosuria.

The influence of parathyroid extract on the glucose tolerance test in diabetes was studied. Eleven cases suffering from diabetes of varying degrees of severity were chosen. The procedure was as follows: each patient after fasting fourteen hours received 100 grams of glucose in the form of a 50 per cent solution. Four blood specimens were taken: one, a fasting level immediately before ingestion and three at the following periods, forty-five minutes, two hours, and three hours after ingestion. During this period the urine was collected and its glucose content determined by Benedict's method. The estimation of the blood sugar was by the Kramer-Gittelman[18] method in which blood is obtained by pricking the finger tip. It has been shown by Foster[19] that the sugar content of such blood is identical with that derived from the radial artery; in other words blood from the finger tip may be considered arterial blood. Two days later a similar procedure was carried out. In the interval between the tests parathyroid extract-Collip (Eli Lilly & Co.) was given. The dosage and time of administration are indicated in Table I. Some of the cases received calcium lactate by mouth in addition to the parathyroid extract. An analysis of the results (see Table I) reveals the following:

a. Fasting level.—In the first eight cases the fasting level blood-sugar concentration of the second curve is lower than that of the first; in the last three, however, the reverse is true.

TABLE I

CASE NO.	1		2		3		4		5		6		7		8		9		10		11	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Fasting level	177	148	249	161	172	162	243	185	207	162	186	145	289	228	395	280	181	190	218	245	184	220
45 minutes posteibum	333	279	392	271	266	252	360	333	331	241	314	233	444	335	574	359	266	271	351	358	261	310
2 hours posteibum	337	339	423	444	333	317	463	444	317	187	249	224	522	392	670	520	274	299	404	475	279	320
3 hours posteibum	353	327	375	383	337	325	389	380	228	277	186	138	467	280	434	530	199	242	293	265	248	296
Urine glucose for three hour period	10.3	12.8	17.5	21.0	9.6	8.6	–	–	13.4	8.6	13.6	16.4	–	–	31.5	18.0	8.0	8.0	12.5	12.5	8.0	6.3

Urine Glucose expressed in grams.
Blood sugar in milligrams per 100 c.c. of blood.
Cases 1 and 2: 10 units of parathyroid extract-Collip 16 hours before second glucose tolerance test.
Cases 3, 4, and 5: 10 units of parathyroid extract-Collip 16 hours before second glucose tolerance test.
5 units of parathyroid extract-Collip 1 hour before second glucose tolerance test.
Cases 6, 7, and 8: 10 units of parathyroid extract-Collip 16 hours before second glucose tolerance test.
5 units of parathyroid extract-Collip 1 hour before second glucose tolerance test.
In addition, 20 grains calcium lactate three times daily in the interval between tests.
Cases 9, 10, and 11: 15 units of parathyroid extract-Collip 16 hours before second glucose tolerance test.
5 units of parathyroid extract-Collip 1 hour before second glucose tolerance test.
A = control curve.
B = curve after administration of parathyroid extract.

b. This is also true of the forty-five minute posteibum blood-sugar concentration.

c. In the two-hour posteibum blood-sugar concentration one case shows no change; in four, that of the second curve is higher than that of the first, and in six, the reverse is true.

d. In the three-hour posteibum blood-sugar concentration two cases show no change; in five, that of the second curve is higher than that of the first, and in four, the reverse is true.

One is forced to conclude that parathyroid extract does not appreciably influence the blood-sugar curve.

e. Glycosuria.—Three cases show an increase in glycosuria after parathyroid administration, four a decrease, and two no change.

SUMMARY AND CONCLUSIONS

The literature on the relationship of calcium to diabetes mellitus and of the parathyroids to diabetes and carbohydrate metabolism in general has been revived. One would gather from the work of Cammidge and Howard[13, 14] and others that although by itself parathyroid extract has no influence on carbohydrate metabolism, it distinctly augments the potency of insulin. The administration of glucose causes a secretion of insulin into the circulation. The glucose tolerance test may be considered an index of the amount of insulin secreted. If parathyroid extract increases the potency of insulin, this should make itself evident in a reduction in the height and length of the blood-sugar curve and in the amount of glycosuria. We did not find this to be the case. Our results are completely at variance with those of Cammidge and Howard and others. If we are to assume that parathyroid extract-Collip constitutes the sole active principle of the parathyroids, then we are forced to conclude that the latter are not concerned with carbohydrate metabolism.

We are indebted to Dr. Max Lederer, director of the laboratory, for helpful suggestions, and to Dr. Edmund Shlevin for the use of the cases in his clinic.

REFERENCES

[1]Wishnofsky, M.: Studies in Calcium and Carbohydrate Metabolism. I. Calcium and Glucose Tolerance in Diabetes Mellitus, Jour. Lab. and Clin. Med., 1927, xiii, 133-138.

[2]Von Moraczewski, W.: Stoffwechselversuch bei Diabetes Mellitus, Centralbl. f. inn. Med., 1897, xviii, 921-932.

[3]Von Moraczewski, W.: Stoffwechselversuch bei Diabetes Mellitus, Ztschr. f. klin. Med., 1898, xxxiv, 59-65.

[4]Von Noorden, C.: Metab. and Pract. Med., 1907, iii, 600.

[5]Falta, W., and Whitney, J. L.: Zur Kenntnis des Eiweiss- und Mineralstoffwechsels pankreatischer Hunde, Beitr. z. chem. Phys. u. Path., 1908, xi, 224-228.

[6]Kahn, M., and Kahn, M. H.: Lime Deficiency of Diabetes Mellitus, Arch. Int. Med., 1918, xviii, 212-227.

[7]Davies, D. T., Dickens, F., and Dodds, E. C.: Observations on Preparation, Properties, and Source of Parathyroid Hormone, Bio-Chem. Jour., 1921, xx 695-702.

[8]Rothchild, F., and Jacobsohn, M.: Der Einfluss von Insulin auf die Blutzusammensetzung beim Diabetes Mellitus, Ztschr. f. klin. Med., 1926, civ, 70-79.

[9]Harrop, G. A., and Benedict, E. M.: The Participation of Inorganic Substances in Carbohydrate Metabolism, Jour. Biol. Chem., 1924, lix, 683-697.

[10]Collip, J. B.: The Calcium Mobilizing Hormone of the Parathyroid Glands, Jour. Am. Med. Assn., 1927, lxxxviii, 565.

[11]Eppinger, Falta, and Rudinger: Quoted in Barker's Endocrinology and Metabolism, 1922, i, 635.
[12]Marine: Quoted in Barker's Endocrinology and Metabolism, 1922, i, 635.
[13]Cammidge, P. J., and Howard, H. A. H.: Interrelation of Insulin and other Gland Extracts in Metabolism, Jour. Metab. Research, 1924, vi, 189.
[14]Cammidge, P. J., and Howard, H. A. H.: Influence of Insulin upon Activity of Diastatic Ferment of Liver, Jour. Metab. Research, 1925, v, 95-105.
[15]Winter, L. B., and Smith, W.: On a Possible Relation between the Pancreas and the Parathyroids, Jour. Physiol., 1923, lviii, 108-110.
[16]Forrest, W. D.: Effect of Parathyroid on Blood Sugar Curve After Insulin, Brit. Med. Jour., 1923, ii, 916-917.
[17]Petty, O. H., Stoner, W. H., and Schaffer, H. W.: Clinical Application of Parathyroid Extract, 1926, xlii, 173-182.
[18]Kramer, B., and Gittelman, I. F.: Technic for Quantitative Estimation of Glucose in Small Amounts of Blood, Jour. Am. Med. Assn., 1923, lxxxi, 1171.
[19]Foster, G. L.: Some Comparisons of Blood Sugar Concentration in Venous Blood and in Finger Blood, Jour. Biol. Chem., 1923, lv, 291-301.

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NEW IODO DERIVATIVES OF PHTHALEINS*

By Frederick R. Greenbaum, D.Sc., Philadelphia, Pa.

HALOGENATED phthaleins have recently aroused a great deal of interest. They have been introduced for cholecystography by E. A. Graham and his coworkers.[1] He investigated tetraiodophenolphthalein and tetrabromophenolphthalein. Today tetraiodophenolphthalein as a sodium salt, tetrabromophenolphthalein, and tetrachlorphenolphthalein are prepared and marketed by a number of American manufacturers. They are being used in cholecystography.[2]

The very unpleasant side reactions that occur after intravenous injection of these compounds show that they are still very toxic, and hence a search for new iodine compounds in this series of phthaleins was indicated. The iodine compounds were preferably synthetized on account of the high atomic weight of iodine and for the purpose of cholecystography was absolutely essential to make these drugs efficient.

Previous to this time, halogenated phthaleins have been considered important from two points of view: first as indicators, and second for spectra absorption. Tetrabromophenolphthalein and tetrabromofluorescein were studied by D. L. Pratt, F. B. Doam, and A. W. Harvey.[3]

For this investigation, the two different isomers of tetraiodophenolphthalein, the α-tetraiodophenolphthalein and then the β-tetraiodophenolphthalein were prepared. Then a completely saturated iodine compound of phenolphthalein was synthetized, namely, octoiodophenolphthalein, which as far as I am aware has not been prepared before. This compound should prove of immense interest in cholecystography as it has the highest molecular weight of them all. Its exceedingly high content of iodine should prove very advantageous in this field.

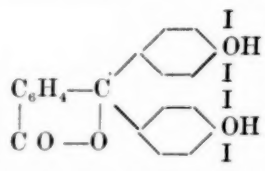
*Received for publication, February 22, 1928.

The introduction of iodine was accomplished by several methods. Phediodo-dinitro-phenolphthalein, and iodine introduced into dinitrofluorescein gave di-iodo dinitro-fluorescein. Metacresolphthalein gave diodo-metacresolphthalein. Rhodamine B gave by one method a mono-iodo rhodamine B, and by using another method a diodo-rhodamine B was obtained. Thymolphthalein gave when treated with iodine a mono-iodothymolphthalein, but no diodo compound could be prepared.

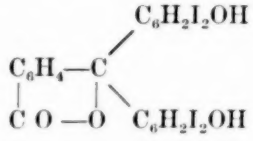
The introduction of iodine was accomplished by several methods. Phenolphthalein is the phthalein which is the easiest to halogenate as the higher homologues often require more energetic methods used than the one in the case of phenolphthalein. The iodo compounds obtained were analyzed for iodine by the Carius' method.

EXPERIMENTAL PART

α *tetraiodophenolphthalein* of the following structural formula was prepared by the well-known method of Classen and Loeb.[4]

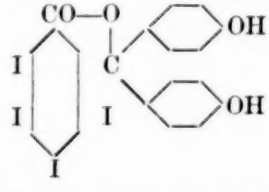


It is an amorphous white powder, insoluble in cold or hot water and insoluble in acids, soluble, however, in hot ethyl alcohol, in ether and other organic solvents, and soluble in dilute alkalies with a blue color. This color disappears on the addition of more alkali. The compound decomposes at 220° C. giving off vapors of iodine. The compound



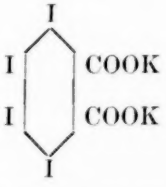
analyzed for iodine gave 61.6 per cent, and calculated to contain 61.8 per cent of iodine.

In order to prepare the isomers to the above compound, namely, the β-tetraiodophenolphthalein, the structural formula of it looks as follows:

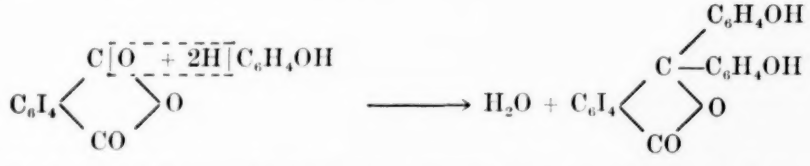


It contains the four atoms of iodine in the phthalic acid ring, while the alpha compound contains the iodine in the phenolic rings. It was necessary to first prepare tetraiodophthalic acid anhydride, and then to fuse this compound with phenol to form β-tetraiodophenolphthalein.

Tetraiodophthalic acid anhydride was prepared according to D.R.P. 50177, January, 1889,[5] in the following way: 20 g. of phthalic acid anhydride was dissolved in 480 g. of 15 per cent SO_3 containing fuming sulphuric acid, and was heated to 90° to 100° C. Then 80 g. of iodine was gradually added and the temperature raised to 18° C., and kept there until the reaction was over. It was then allowed to cool, the acid was decanted, washed with sodium bisulphite, filtered and washed with SO_2 containing water to remove free iodine, and recrystallized from hot glacial acetic acid. The compound obtained represented a yellow prism with a melting point of 134° C. It is insoluble in water and organic solvents, soluble in hot glacial acetic acid and also soluble in hot potassium or sodium hydroxide under formation of the potassium or sodium salt of tetraiodo phthalic acid of the following formula:



In order to prepare now the β-tetraiodophenolphthalein, the tetraiodophthalic acid anhydride is fused with phenol in the following manner: 1 mol. of tetraiodophthalic acid anhydride and 2 mol. of stannic chloride are heated in an oil bath to a temperature of 140° C. for several hours. At first the compounds begin to melt, but soon under the dehydrating influence of stannic chloride a chemical reaction takes place, which is evident by the change in color from a light yellow to a pink, to a red, and finally to a dark red. A combination occurred according to the following equation:



The dark red fusion obtained was extracted after cooling with hot water to dissolve the stannic chloride. The residue is dissolved in warm dilute (15-20 per cent) sodium hydroxide solution, and this pink or reddish solution is filtered off from the insoluble portions which consist of unchanged tetraiodophthalic acid anhydride. The filtered solution is acidified with dilute hydrochloric acid, filtered, washed, redissolved in sodium hydroxide, acidified again and filtered, and washed with hot water until free from acid and dried. The analysis gave 57.75 per cent of iodine and calculated, for $C_{20}H_8O_4I_6Na_2$ = m.w. 866, should be 58.6 per cent of iodine.

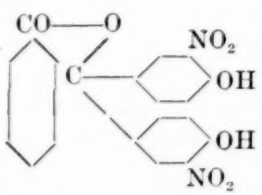
The free β-tetraiodophenolphthalein represents an amorphous powder insoluble in cold or hot water, insoluble in all common organic solvents, but soluble in alkalies with a pink to a reddish color which disappears on addition of strong alkalies. The a-tetraiodophenolphthalein gives a blue color with dilute alkalies.

From this β-tetraiodophenolphthalein, the *octoiodophenolphthalein* was prepared as follows:

Twenty gm. of b-tetraphenolphthalein was dissolved in excess alkali, and to this solution 72 g. of iodine which was dissolved in 84 g. of 20 per cent sodium hydroxide solution was added under stirring and heated to boiling. Then it was gradually acidified by adding in a fine stream glacial acetic acid, under constant stirring and heated to a boil for one-half hour, after this time it was neutralized with sodium hydroxide solution and then rapidly acidified with hydrochloric acid. It was again heated to a boil, allowing the supernatant solution to settle, decanted, and then filtered off the precipitate and then washed with water until free from acid. The precipitate was redissolved in sodium hydroxide, sodium bisulphite added to the solution and acidified which precipitated the iodo compound and at the same time liberated sulphur dioxide from the sodium bisulphite, this removes any free iodine included in the precipitate of octoiodophenolphthalein. The precipitate was then filtered off, washed thoroughly with water until free from acid or any other impurities, and dried in vacuum desiccator to constant weight. The analysis gave 76.25 per cent for iodine and calculated for $C_{20}H_6O_4I_8$ (m.w. 1326) the iodine content should be 76.60 per cent.

Detoiodophenolphthalein is a yellow crystalline substance insoluble in cold or hot water easily soluble in dilute alkalies, the solution having a faint greenish color. This substance on account of its high molecular weight (1326) and its high content of iodine 76.25 per cent is particularly suitable for experimentation in cholecystography, and the attention of physicians working along these lines should be directed to this new and very interesting iodophthalein.

The next iodophthalein prepared used as a starting material dinitrophenolphthalein. Dinitrophenolphthalein was prepared according to the German patent in the following way: 100 g. of phenolphthalein was dissolved or suspended in 100 c.c. of glacial acetic acid, and is then gradually treated, under cooling and stirring, with a mixture of 70 parts of nitric acid (specific gravity 1.48) and 210 parts of concentrated sulphuric acid, care being taken that the temperature remains at 20° C. The liquid after all the acid has been added gets slightly warm and on cooling and standing a yellow crystalline nitro compound of phenolphthalein is precipitated, which is filtered and washed. It is then recrystallized once from boiling glacial acetic acid and represents dinitrophenolphthalein.



Dinitrophenolphthalein is a yellow crystalline substance, melting point 196° C., insoluble in cold or hot water, soluble in sodium hydroxide with an

orange color, and can be reprecipitated with an acid. It is slightly soluble in ethyl alcohol and methyl alcohol, slightly soluble in ether, easily soluble in hot glacial acetic acid from which it can be recrystallized. The analysis for nitrogen gave 6.75 per cent and 6.93 per cent, and calculated for $C_{20}H_{12}O_8N_2$ the nitrogen amounts to 6.86 per cent.

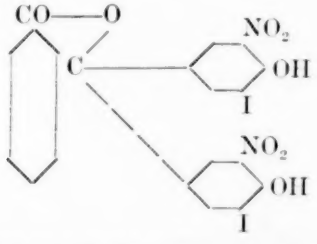
This pure dinitrophenolphthalein was used for the introduction of iodine which resulted in the formation of *diododinitrophenolphthalein:*

Forty grams of dinitrophenolphthalein was dissolved in 40 g. of 20 per cent sodium hydroxide and 300 g. of water. This solution was boiled and filtered. Then 120 g. of unsublimed metallic iodine was dissolved in 140 g. of 20 per cent NaOH solution and 300 g. of water. The two solutions were now poured together, and 125 g. of glacial acetic acid was added slowly under stirring. This addition of acetic acid caused the liberation of iodine in a finely suspended state, and the substitution now takes place. It was heated to a boil and kept boiling for about one-half hour. Then 85 g. of 20 per cent NaOH solution was added to neutralize the acetic acid, and then rapidly acidified with 125 g. concentrated hydrochloric acid and 125 grams of water, boiled for a short time, allowed to settle, the supernatant iodine solution was decanted, the precipitate was washed with hot water, filtered and washed again with water until the wash water was almost colorless. Then the precipitate was dissolved in dilute sodium hydroxide, filtered, and precipitated with concentrated hydrochloric acid. This precipitate was filtered off and redissolved in NaOH, reprecipitated and washed free from HCl. If necessary, sodium bisulphite was added before acidifying it, and then HCl added; this removes the free iodine present. This yellow precipitate was then thoroughly dried on a steam-bath and then in a desiccator.

The analysis for nitrogen using the Kjeldahl reduction method and for iodine using Carius' method gave the following results:

calculated for $C_{20}H_{10}O_8N_2I_2$		
for nitrogen	4.2	per cent
found for nitrogen	4.3	per cent
calculated for iodine	38.4	per cent
found for iodine	38.21	per cent

So that the formation of diododinitrophenolphthalein was established, and would have the following structural formula:

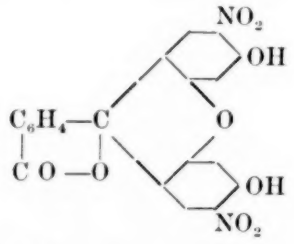


Diododinitrophenolphthalein is a light yellow amorphous powder with a melting point of 249° to 250° C., insoluble in cold and hot water, soluble in NaOH

with an orange color, insoluble in the usual organic solvents, soluble in glacial acetic acid. No more iodine can be introduced as all the ortho positions are occupied.

The next higher homolog of phenolphthalein, namely, fluorescein was nitrated according to Beilstein.[6] One part of fluorescein was dissolved in 10 parts of concentrated sulphuric acid, kept at 0° C. and 2 parts of concentrated fuming nitric acid was added, then poured on ice and filtered, the precipitate was washed, redissolved in methyl alcohol, filtered and precipitated in H_2O, filtered and dried.

The analysis for nitrogen gave 6.51 per cent, while calculated for $C_{20}H_{10}O_9N_2$ gave for nitrogen the value 6.6 per cent. So that the dinitrofluorescein of the following formula was obtained.



It represents a yellowish orange powder, soluble in sodium hydroxide with a reddish-blue tint, insoluble in cold or hot water, and insoluble in organic solvents.

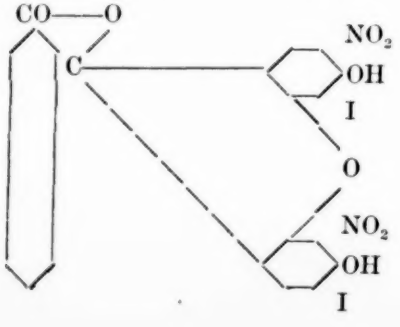
The introduction of iodine into dinitrofluorescein gave a new derivative; e.g., the dinitrodiodofluorescein, which was prepared in exactly the same way as the diododinitrophenolphthalein.

This method of introduction of iodine as described above proved satisfactory in all fluorescein derivatives and in many other phthaleins.

The diododinitrofluorescein, when analyzed yielded for $C_{20}H_8O_9N_2I_2$

for nitrogen calculated	4.1 per cent
for nitrogen found	4.1 per cent
for iodine calculated	37.7 per cent
for iodine found	37.1 per cent

So that the following structural formula must be assigned to this compound:

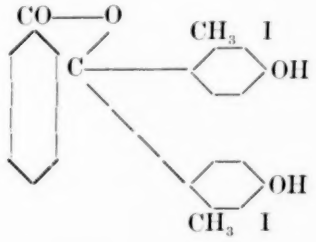


It is an orange-red amorphous powder, insoluble in cold and hot water, soluble in ethyl and methyl alcohol, and soluble in ether, soluble in caustic soda solution with a blue color, and on acidification the diododinitrofluorescein is precipitated.

Metacresolphthalein is a yellowish-brown amorphous powder which was prepared by fusing meta cresol and phthalic acid anhydride together in the same way as metacresolphthalein is prepared according to Beilstein.[7] Metacresolphthalein is soluble in dilute NaOH giving a purple color, and is precipitated with mineral acids. It is soluble in ethyl alcohol and methyl alcohol, and all common organic solvents, in concentrated sulphuric acid it dissolves with a brownish color.

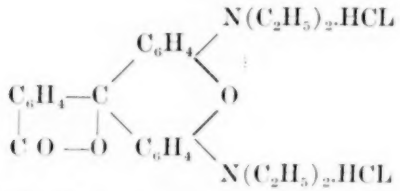
From metacresolphthalein an iodo compound was prepared and proved to be *diodometacresolphthalein* in the following manner: 10 g. of metacresolphthalein was dissolved in an excess of caustic soda. This solution was filtered and 20 g. of iodine and 20 g. of potassium iodide dissolved in 100 c.c. of water were added gradually under stirring for two hours, acidified with hydrochloric acid, filtered and washed, redissolved in sodium hydroxide, and reprecipitated with HCl. This procedure was repeated twice, then filtered and washed with water to free from HCl, and dried on a steam-bath and analyzed.

The analysis calculated for $C_{22}H_{16}O_4I_2$ gave for iodine 42.4 per cent, and the iodine found was 42.0 per cent. Therefore, the formula of this compound looks as follows, with a molecular weight of 598.



Diodometacresolphthalein is a brown amorphous powder, melting point of 214° C., insoluble in cold and hot water, insoluble in ether, somewhat soluble in ethyl and methyl alcohol, very slightly soluble in dilute sodium hydroxide, it dissolves to form a dark brown solution. It is interesting to note that this exhaustive method for the introduction of iodine does not furnish a tetraiodometacresolphthalein, as would be expected, but only a diodometacresolphthalein in spite of the fact that the four ortho positions are according to the above formula not occupied.

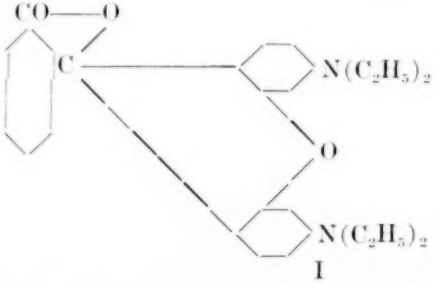
The next compound tried was rhodamine B. The commercial compound available is the hydrochloride of tetraethyldiaminophenolphthalein of the following formula:



This rhodamine B was treated by two methods for the introduction of iodine. The direct addition of a solution of iodine and potassium iodide to an alkaline solution of rhodamine B resulted in a monoiodorhodamine as the analytical figures show:

calculated for nitrogen	4.90 per cent
found for nitrogen	4.60 per cent
calculated for iodine	23.30 per cent
found for iodine	22.70 per cent

So that the compound obtained was a monoiodo compound of this formula:



I

It is of course clear that this is an unsaturated compound, but every attempt to introduce more iodine by the use of this method failed. At last the following method was tried and resulted in a diodorhodamine B: 2.2 g. of highly purified rhodamine B was dissolved in 200 c.c. of water, and to this solution 2.3 g. of iodinetrichloride dissolved in 100 c.c. of water containing 10 c.c. of concentrated hydrochloric acid was added. Immediate precipitation of a reddish flocculent compound occurred. This was filtered off and washed with water until all the acid was removed, then dried and analyzed. The analysis shows:

Calculated for nitrogen (for a diodo compound)	4.0 per cent
found for nitrogen	3.9 per cent
calculated for iodine	36.4 per cent
found for iodine	36.3 per cent

So this undoubtedly shows that the compound obtained was di-iodo-rhodamine B.

It represented a reddish-brown powder, somewhat soluble in cold water and hot water, insoluble in dilute alkalies, insoluble in acids, very soluble in ether, in methyl and ethyl alcohol. The low solubility of this compound is undoubtedly a disadvantage for medical purposes.

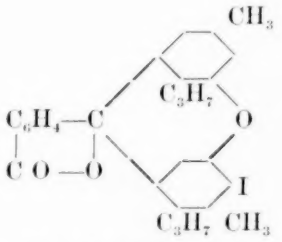
The last phthalein which was included in this study was thymolphthalein. Eight and six-tenths grams of thymolphthalein, obtained from the Eastman Kodak Co., was dissolved in 8.0 g. of 20 per cent sodium hydroxide solution,

and 60 g. of water, boiled, and to this solution 24 grams of iodine which was dissolved in 28 g. of 20 per cent sodium hydroxide was added. Then 60 grams of water was added, boiled for half an hour and acetic acid added, boiled again, neutralized with NaOH, and acidified with HCl, boiled, filtered, purified, and dried on a steam-bath and in a desiccator.

In spite of this vigorous iodine introduction method only a monoiodo compound resulted, and attempts to use iodinetrichloride and iodinemonochloride resulted in the same monoiodocompounds as the analysis shows. For $C_{28}H_{27}O_3I$

iodine calculated	23.70 per cent
and for iodine found	23.96 per cent

So that the obtained compound was monoiodothymolphthalein of the following formula:



Monoiodothymolphthalein is a brown amorphous powder insoluble in cold or hot water, soluble in common organic solvents, soluble in alkalies with a blue color.

Table I compiles the analytical figures of the prepared iodophthaleins as well as their molecular weights, as the high molecular weight and the iodine content are of the utmost importance for their use in cholecystography.

A glance at this table will show that from the point of view of high content of iodine and high molecular weight the best compound is undoubtedly octoiodophenolphthalein with an iodine content of 76.6 per cent and a molec-

TABLE I

SHOWING THE ANALYTICAL FIGURES OF PREPARED IODOPHTHALEINS

NAME OF PHTHALEIN	ELEMENTS ANALYZED	THEORY	FOUND	MOLECULAR WEIGHT
α-tetraiodophenolphthalein	I	61.8	61.6	822
β-tetraiodophenolphthalein (Na salt)	I	58.6	57.75	866
Octoiodophenolphthalein	I	76.6	76.25	1326
Diododinitrophenolphthalein	N	4.2	4.3	660
	I	38.4	38.21	
Diododinitrofluorescein	N	4.1	4.1	674
	I	37.7	37.1	
Diodometacresolphthalein	I	42.4	42.0	598
Monoiodorhodamine B	N	4.90	4.60	567
	I	23.30	22.70	
Diodorhodamine B	N	4.0	3.90	694
	I	36.4	36.3	
Monoiodothymolphthalein	I	23.70	23.96	536

ular weight of 1326. Next to this compound are the alpha and beta tetraiodophenolphthaleins, so that a pharmacological study of octoiodophenolphthalein and β-tetraiodophenolphthalein seems highly advisable.

REFERENCES

[1]Graham, E. A., and Copher, G. H.: Cholecystography, Jour. Am. Med. Assn., 82, May 3, 1924; Cholecystography, Jour. Am. Med. Assn., 84, Jan. 3, 1925; Cholecystography, Jour. Am. Med. Assn., 84, April 1925, p. 1175.
[2]New and Non Official Remedies 1927 pps. 164-167.
[3]Pratt, D. L., Doam, F. B., and Harvey, A. H.: Jour. Am. Chem. Soc. xli, II, p. 1293.
[4]Classen, Alex. and Loeb, W.: Berichte der Deutch Chem. Ges. 28, 1895, ii, 1610.
[5]D.R.P 50177: January 1889, Friedlander, i, p. 93.
[6]Beilstein: 1896, ii, 2060.
[7]Beilstein: 1896, ii, 1897.

GINGIVITIS, II

The Clinical Appearance of the Gingivae in Different Forms and Stages*

By Robert A. Keilty, M.D., Washington, D. C.

THE purpose of this paper is to present the physical characteristics of inflammatory changes in the gingivae, as they were observed in the composite picture of a study of 5,000 cases as previously reported.[1] My ideas on gingivitis have been embodied in that report, and the present paper is one of a series giving the details of different phases of the subject.

The older terms for inflammatory, exudative and ulcerative changes in the gums, such as Rigg's disease and pyorrhea have been discarded in this study. The long cumbersome terms, such as "subgingival osteoperiosteal periodontitis" have also been set aside in favor of the shorter and equally inclusive term "gingivitis." Just as the term "pulmonary tuberculosis" includes all forms of tuberculosis in the lung in which the pleura, alveoli, bronchi, and bronchioles are involved and "tonsillitis" all changes on the surface, in the crypts, in the follicles, and in the retrotonsillar solitary glands of the tonsil, so there is included under the term "gingivitis" all degrees of pathology involving the soft structures of the gums from minor to advanced stages including periodontitis or the changes in the tooth socket. For the sake of the purist there is no objection to the addition of the term periodontitis when there is x-ray evidence of bone involvement in the chronic cases of gingivitis. Such a terminology would then be gingivitis with periodontitis or gingivo-periodontitis. This seems unnecessary when it is definitely understood that gingivitis includes all of these advanced changes. At any rate pyorrhea should be dropped from the literature. It is too firmly fixed to the condition of suppurating gums and loose teeth and can never be made

*From the Laboratories of The Diagnostic Center, U. S. Veterans Bureau, Washington, D. C.
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to include the recognition of the early changes in the gums which are far more important in the application of future prophylactic measures.

THE NORMAL GUMS

The gingivae are soft structures surrounding the necks of the teeth and covering the bony jaws. There is a modified stratified squamous epithelial covering backed up by a corium of loose connective tissue, rich in blood vessels, lymph channels, and submucosal glands. The surface of the gums normally does not join the necks of the teeth at a sharp angle but around each tooth is a small anatomic sulcus. The actual attachment of the gum is slightly below the general surface with the result that a small circular anatomic ditch surrounds each tooth. According to Gottlieb the detachment normally never reaches the cemento-enamel junction but is always above it. The reason for its being considered lower before 1920, it seems to me, was due to the fact that pathologic conditions about the gingivae being so numerous, a real histologic normal is rarely seen in the adult.

The union of this surface epithelium at the bottom of gingival pockets, with the peridental investiture of the tooth and the beginning of the periosteum of the tooth socket, is a very vulnerable point. At the same time this shows a very variable histologic structure, as has just been stated, not because it is variable anatomically but because there are present in most every mouth, even at an early age, pathologic changes at this point which, of course, give variable histologic changes. The interpretation of these changes from the purely histologic standpoint is responsible for much of the discussion in dental literature as to just what constitutes the normal. The interpretation should be made with the pathology as well as the histology in mind. Suffice it to say that the gingival sulcus when present pathologically, offers one of the most vulnerable points in the body protective mechanism and pari passu, one of the most frequent portals of entry for invasion with microorganisms.

In the attachment of the perfectly normal gums, which are rarely seen, to the teeth an anatomic sulcus is not grossly apparent. A 22-gauge wire will not catch on any shelf formed by the sulcus nor penetrate any appreciable distance between the gums and the teeth. When this shelf can be seen or a sulcus is present some pathology has taken place.

The gums normally are not red but are quite pallid or slightly pink. There is no bogginess so that pressure, unlike the case of the lips, does not cause a pallor with a sharp return of capillary blood. While the gingivae in the normal are supplied with a rich capillary network, this is not as apparent grossly as in other mucous membranes of the mouth. The surface of the gums is smooth, not glazed, and presents no granularity. Such normal mouth is occasionally seen but is very rare. Any deviation from this normal constitutes an evidence of pathologic change, the cause of which should be investigated carefully. The best examples of normal gums are seen from 18 to 25 years of age after the trauma of dentition has subsided and before the neglect of teeth has started. On the other hand some of the worst mouths are seen during these ages. It is very rare to see a perfectly normal mouth after the third decade.

TYPES OF GINGIVITIS

Every mouth is an individual study and subject to conditions peculiar to the individual. No two mouths are exactly alike but in the composite picture certain types of pathologic changes in the gums present definite groups. Gingivitis being an inflammation may be fundamentally divided into acute, subacute, and chronic forms. The specific inflammatory diseases, tuberculosis and syphilis, are not included in this study. The gums are markedly influenced in both of these diseases. This will be the subject of a separate report. All of the phenomena of acute and chronic inflammation of a mucosal surface appear in the gums and contiguous structures and even involve the teeth. Thus rubor, calor, tumor, and dolor constitute the fundamentals of acute gingivitis, and exudation, suppuration, and ulceration represent subacute and chronic gingivitis. While strictly speaking, there is no pathologic entity representing a subacute inflammation, the combination or progression of an acute and chronic inflammation is certainly well seen clinically in the gums.

ACUTE GINGIVITIS

Acute changes in the gums may be localized to the area about single teeth, when they are often traumatic in origin. These include examples of traumatic occlusion and may or may not be complicated by other etiologic factors, infections, operative procedures, dentures, crowns, etc. The vast majority of acute cases are infectious in origin and involve the entire gingivae both upper and lower, central, lateral right or left, and with degrees of severity. This is exactly the same as in tonsillitis, the involvement here being one tonsil, both tonsils, pillars, fauces or uvula.

In gingivitis the acute process is exudative and necrotic with suppuration and false membrane. This is seen in trench mouth and in the acute flareup of a chronic gingivitis following, for example, the extraction of a tooth. The gums are swollen, tender, red, congested, boggy, pit and blanch on pressure. The anatomic ditches separate from the teeth and are filled with the products of exudation. The slightest touch produces hemorrhage. The teeth are covered with sordes (products of the exudate). The gingival surface, on the fifth to the tenth day of the disease in very severe cases, may be covered with a false membrane which is easily stripped off leaving a bleeding necrotic and ulcerated surface beneath. This often becomes so intensive that the entire gingivae and sides of the cheek, fauces, and tonsils are involved as a stomatitis. The mouth is opened with difficulty and in fatal cases this goes on to cellulitis of the entire face and neck.

All of these changes in degree will be seen in an individual case depending upon the severity of the infection, the immunity reaction of the patient, the time of the infection, and the efficacy of the treatment. Acute gingivitis is accompanied by pain which is often exquisite, and tenderness not only in the gums but the whole face. The patient looks sick and feels bad, the result of systemic infection. The breath is sweetish, offensively foul due to the necrosis and to rapid bacterial reproduction with gas formation. Most of the foul breaths so often attributed to sour stomach and biliousness, if not

due to a decaying tooth, are due to gingivitis. This is proved by the characteristic odors of culture tubes from gingival bacterial flora and by the prompt disappearance of breath odors when adequate local chemotherapeusis is carried out. While this is the picture of "trench mouth," an acute, exudative and suppurative gingivitis, it is not an entity but a part of the general scheme of gingivitis. Acute gingivitis is secondarily divided for classification into traumatic, exudative, suppurative, necrotic, ulcerative, and membranous forms.

SUBACUTE GINGIVITIS

Subacute gingivitis without a definite pathology does not represent a large group of cases. The group is necessitated by the patients who show subjectively and objectively basic chronic changes with varying degrees of acute flareups. These, I believe, are due to definite cycles of the infectious organisms involved. These organisms at different periods in their growth represent sexual and asexual stages varying in their toxicity and thus responsible for the acute reactions on the part of the tissue. Reinfection also plays an important part in these subacute cases. The universal custom of kissing and the direct chances for contact is without doubt the modus operandi and one of the important reasons for the widespread incidence of gingivitis.

The picture of the gums in subacute gingivitis varies within wide limits. It would seem best to restrict this class to cases with mild pictures of chronic changes. These show localized or focalized tender areas, where the gums are unduly boggy. In these areas the trenches are deep, almost pockets, with heavy exudate while the rest of the mouth is more or less quiescent. This group may also include some of the early insidious chronic cases without any great activity. There are frequently evidences of exudation and infection. These cases are often painless and symptomless and the gums are said to be "healthy red looking." Under the same conditions the acute cases which have subsided and are becoming early chronic may also be classed under the subacute groups. Subacute gingivitis is secondarily divided for classification into exudative, reinfected, focalizing, and early chronic.

CHRONIC GINGIVITIS

The group under the classification of chronic gingivitis is by far the largest and most of us fall into it. Over 90 per cent of the 5,000 cases studied presented some form of gingivitis and this represents, I believe, a cross-section, at least, of the people of this country. The large group of cases studied were mostly residents in the central part of Pennsylvania. During the study I was a little fearful that the results might be a more or less local state of affairs, but in the past eight months I have seen several hundred cases in Washington, D. C., and have found exactly the same conditions and incidence of occurrence. The Washington cases represent a more selected group, having had excellent dental care, in spite of which gingivitis had continued to progress.

Chronic gingivitis presents a picture much more varied than either of the other two forms. This is due almost entirely to the stage of progression of the disease. It would seem well to first divide them into groups of mild, moderate and advanced types. There is no sharp line between these cases,

all show some of the fundamental changes; but the question of severity of these fundamental changes, length of time they have developed, and the amount of deep bone change, determine their grouping. The question of the bacteriologic flora in a given case plays a very important rôle in the study of that individual case and this relationship will be discussed in another paper. So far we have been unable to classify gingivitis on a basis of its different types of bacterial infection and protozoal infestation, but accumulation of data may provide in the future a more definite classification from this point of view.

All cases of chronic gingivitis should have a complete dental x-ray and those showing periodontal changes about one or more teeth (now almost universally diagnosed as pyorrhea) should be classed as advanced chronic gingivitis or gingivoperiodontitis. All such deep changes will be accompanied by marked changes in the soft structures. The reverse is not true. Severe and advanced chronic changes may appear in the gingivae without x-ray evidence of deep periodontal change.

The surface of the gums in chronic gingivitis is rarely smooth and glistening but is usually glazed and granular. This granularity is often very marked. Some gingivae have little blood vessel reaction, especially the long-standing chronic fibrous forms and are pallid white, others vary through pink and red to deep purplish blue. I have seen the blueness of passive congestion so marked that chronic lead poisoning had been suspected. All chronic cases have some degree of swelling, not the boggy edema of the acute cases but the firmer, more tense edema of passive congestion. Under appropriate treatment gums may subside over a half their thickness. This swelling is mostly fluid but in the advanced cases much cellular reaction with fibrosis of the subgingival mucosa has resulted. Some of the moderate cases and many of the long-standing cases show a ridging along the gingival margins often associated with recession so that the ridge is a half moon. This is always a subgingival fibrosis and the result of organization fibrogenic reaction against infection.

Gingival recession occurs to some degree even in the mild cases but more often is a part of the advanced changes. It is due to the loss of structure by ulceration and equally as important to the contraction following efforts at repair. All of the successfully treated cases will show more recession after treatment than before. The elimination of sulci physically results in retraction of the overlapping gingivae and exposure of dentine. Such patients will often consider that they are worse, the exposed dentine is more tender, the gums have receded and more of the teeth are exposed. They may even say their teeth are elongating or coming out. It is well to explain before treatment that this is an evidence of repair and that in the end no pain or discomfort will result.

Recession may affect a single tooth, a series of teeth as the lower centrals or may be more or less general. It usually involves the labial and lingual surfaces, leaving pyramids of gingival structure between the teeth. Occasionally the reverse is true, marked recession occurring between the teeth with little on the labial and lingual surfaces. This is more apt to be present

in subacute and chronic cases following a destructive, necrotic acute ulcerative gingivitis. Finally recession unassociated with other evidences of gingivitis appears in a small number of cases of tuberculosis and syphilis.

I have stated that the normal gingiva has no macroscopic sulcus or trench at its attachment to the enamel of the tooth which in the normal, as pointed out by Gottlieb, is above the cemento-enamel line. The anatomic ditch is therefore an evidence of gingivitis and where present if only one or two mm. in depth will show some exudate. The same fact may be stated in another way, the presence of actual exudate, no matter how small in amount, in a gingival sulcus is evidence of chronic gingivitis. The so-called anatomic ditch or gingival sulcus in chronic gingivitis varies in depth from one or two mm. to over one cm. where it extends sometimes by direct communication below the root tip of a tooth.

In mild and moderate cases of chronic gingivitis, the sulci are not very deep, usually not involving the periodontal areas, except possibly about one tooth often in the molar areas where for one reason or another the process has become advanced.

The sulci are, generally speaking, about evenly divided about most of the teeth. The more shallow sulci are about the centrals, growing deeper toward the molars. The depth of the pockets increases with the progression of the disease, and the generally deep sulci are a part of the advanced cases.

With the development of sulci in chronic gingivitis exudation from and ulceration of the gingival mucous membrane and submucous connective tissue takes place. The character of this exudate varies within wide limits and will be discussed under the phase of bacterial flora. *It is not necessary to express pus from the margins of the gums to make a diagnosis of chronic gingivitis.* The presence or absence of pus is dependent on several factors and many cases of apparently complete absence of gross pus show a gingival exudate that is microscopically teeming with infection.

One of the main purposes in this study is to call especial attention to the early stages of chronic gingivitis and to urge their recognition. *Intelligent prophylaxis in the early cases will do much to save a future trail of diseases, not the least of which are those of the heart and rheumatic group, and ranks in importance with prophylaxis against the progression of tuberculosis and cancer.*

The patients suffering with chronic advanced gingivitis are usually aware of it. The salty, disagreeable taste of pus, the bad breath odor, the bleeding in the gums, the looseness or loss of teeth are all quite manifest. They have, in addition, usually been told by their dentists that they have "pyorrhea" and many have had several courses of treatments of the various types now in vogue. In spite of all their care their "pyorrhea" is progressing, and they have or have not reached the stage where extraction is necessary.

Chronic gingivitis is secondarily divided for purposes of classification into mild, moderate and advanced as far as the degree is concerned. Exudation, ulceration, recession, and fibrosis cover the gingival pathology while gingivitis wtih periodontitis may be used to cover the cases with x-ray evidence of osseous destruction.

CONCLUSIONS

It is hoped that, in reporting the results of my observations on studies in mouth infections made during the past several years, that the atmosphere will be somewhat clarified and not thrown into any greater confusion as far as the gums are concerned.

The problem of gingivitis as studied grew out of the relationship of mouth infections as etiologic foci in thyroid diseases.

The study of gingivitis has reached certain conclusions that would seem to warrant their detailed report at this time, realizing that an open mind should still be held until this troublesome condition is conquered.

Critical observations of the gums as to appearance have led to the conclusion that the normal gingiva may be said to be almost rare in adults.

The histologic conceptions of the gums in the past must have been based upon the results of pathologic changes and not the perfectly normal relationship.

The histologic anatomist must definitely decide just what constitutes the normal gingival sulcus, and the exact relationship between the soft tissue cells and the enamel of the tooth. This is obviously quite difficult for lack of material from the human mouth.

From the present study it is concluded that any gross evidence of a sulcus associated with exudate, constitutes an early pathology which breaks a line of defense allowing a beautiful culture bed for microorganisms and protozoa. *This initiates a portal of entry which is believed much more frequent and important than root canals.*

A classification of inflammatory changes in the gums for purposes of study and treatment is offered in this presentation. This is based upon the appearances, clinical courses, and pathologic reactions.

The character of the bacteriologic flora and protozoal infestation of the gingiva, sulci, and periodontal osseous structures is most important in the whole study, but so far their relationship cannot be offered as a means of classification of different types of gingivitis.

Gingivitis is worthy of a continued careful and critical study of its complex phases and the laboratory can accomplish a great deal as fixing much of haziness and controversy concerning it.

REFERENCES

[1]Keilty, Robert A.: The Present State of Our Knowledge of Gingivitis, JOUR. LAB. AND CLIN. MED., 1928, xiii, No. 5, p. 451.

A CASE SHOWING UNUSUAL WASSERMANN REACTIONS*

By James J. Short, M.D., and Margaret F. Kelley, A.M., New York City

ALTHOUGH in the overwhelming majority of instances the Wassermann test gives reliable and consistent results, there is an occasional rare instance when results obtained are wholly unreliable. Tabulated results with such a case thoroughly studied in several laboratories are set forth in Table I.

REPORT OF CASE

The patient, S. K., female, aged nineteen was advised to have a Wassermann test because of a paronychia on her finger. The serum was first examined at the Laboratories of the Life Extension Institute and gave complete fixation with each of two antigens. These antigens were obtained from the New York State Department of Health at Albany; one was the plain alcoholic extract of beef's heart, the other the same cholesterolized. Further tests were made when the patient reported a negative result obtained a few days later from the Laboratory of the New York City Department of Health. McNeil's antigens mentioned in the table were obtained from the National Pathological Laboratory and were prepared by extracting hog's heart several times with ether, dessicating, and making up an alcoholic extract from the residue; to this, 0.2 per cent cholesterol was added to form the cholesterolized antigen.

Ordinarily very good agreement between the Board of Health and McNeil antigens has been obtained.

The laboratories indicated in the table to which specimens were sent are New York State Department of Health, Albany; New York City Department of Health, Mr. John Koopman, Director, 505 Pearl Street; National Pathological Laboratory, Dr. Archibald McNeil, Director, 18 East Forty-First Street; Willard Parker Hospital, Sixteenth Street and East River (tests made under supervision of Miss Minnie Wilson); and Laboratory of the late Dr. John A. Fordyce, 8 West Seventy-Seventh Street.

It will be observed from a study of the table that not only was there disagreement between different laboratories, but in several instances varying results in the same laboratory. Varying the fixation period slightly frequently changed the result in our experience. There was a tendency in several tests with the McNeil antigen for the alcoholic antigen to hold more completely than the cholesterolized.

COMMENT

We can offer no explanation for the results obtained in this case other than to say that such instances do occasionally occur. It is questionable if this patient was really syphilitic as her condition would not justify such a diagnosis clinically. Unfortunately we have not been able to follow this patient since these studies were made.

*From the Laboratories of the Life Extension Institute, New York.
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TABLE I

WASSERMANN RESULTS ON BLOOD SERUM S. K.

WASS. NO.	DATE BLED	DATE TESTED	PLACE EXAMINED	FIXATION PERIOD	RESULTS WITH ANTIGENS							
					LIFE EXTENSION INSTITUTE				OTHER LABORATORIES			
					MC NEIL'S		N. Y. S. B. OF H.		ALC.	CHOL.	KAHN TEST	STREPTOCOCCUS FIXATION
					1–150 ALC.	1–300 CHOL.	1–20 ALC.	1–60 CHOL.				
717	3/10/24	3/11/24	L. E. I.	Ice Box 4 hr.			4+	4+				
		3/13/24	L. E. I.	Ice Box 4 hr.			4+	4+				
		3/13/24	L. E. I.	Ice Water Pan 1¼ hr.	–	–						
		3/15/24	L. E. I.	Ice Box 4 hr.	4+	3+	4+	4+				
		3/15/24	L. E. I.	Ice Water Pan 1¼ hr.	–	–						
838	3/21/24	3/22/24	L. E. I.	Ice Box 4 hr.	–	–	±	4+				
		3/25/24	L. E. I.	Ice Box 4 hr.	+ Weak	–	4+	4+				
		3/27/24	L. E. I.	Ice Box 4 hr.	4+	2+	4+	4+				
		3/29/24	L. E. I.	Ice Box 4 hr.	3+	±	+	4+				
		3/24/24	Dr. McNeil	Ice Box 4 hr.					–	–		
		3/24/24		Ice Box Overnight					4+	4+		
		3/24/24	N. Y. C. B. of H. Mr. Koopman						2+		–	
		3/24/24	N. Y. C. B. of H. Miss Wilson								–	
887	3/27/24	3/27/24	L. E. I.	Ice Box 4 hr.	4+	4+	+	4+				
		3/29/24	L. E. I.	Ice Box 4 hr.	2+	–	4+	4+				
		3/28/24	Dr. McNeil						–	±		
		3/29/24	Dr. McNeil						±			
		3/29/24	Dr. Fordyce						–	+		
		3/28/24	N. Y. C. B. of H. Mr. Koopman						+			
		3/28/24	N. Y. C. B. of H. Miss Wilson								–	±
			N. Y. S. B. of H. (Albany) Dr. Gilbert	Ice Box 4 hr.					–	–		
				Ice Box Overnight					Definite fixation	Definite fixation		
930	4/ 1/24	4/ 1/24	L. E. I.	Ice Box 4 hr.	±	±	±	+				
		4/ 3/24	L. E. I.	Ice Box 4 hr.	±	±	–	3+				
		4/ 2/24	Dr. McNeil						3+	4+		
1094	4/ 2/24	4/15/24	L. E. I.	Ice Box 4 hr.	4+	±	–	4+				
		4/17/24	L. E. I.	Ice Box 4 hr.	±	±	±	4+				
		4/19/24	L. E. I.		2+	±	4+	4+				
		4/21/24	N. Y. S. B. of H. Dr. Gilbert	Ice Box 4 hr.					±	±		
			N. Y. S. B. of H. Dr. Gilbert	Ice Box Overnight					3+	3+		

A similar case was recently seen by one of us (J. J. S.). The blood of a young lady suffering with urticaria gave complete fixation with the Board of Health antigens on refrigerating overnight in the ice box. Subsequent specimens were all uniformly negative even after provocative treatment.

McNeil[1] reports an occasional rather strong nonspecific fixation in patients having foci of infection especially with streptococci. It has been our observation in several thousand cases that weak nonspecific fixation (plus-minus) is frequently obtained with cholesterolized antigens in sera from patients giving histories of previous gonorrheal infections. This happens even when no active gonorrheal focus can be found by either clinical or laboratory investigation.

CONCLUSION

While these results show the need for caution in arriving at a diagnosis of syphilis solely from laboratory testimony, it is well to keep in mind that after all the Wassermann test is one of the most accurate and dependable aids in diagnosis that we have. Certainly the ratio of error in this test is far lower than that in the physical examination of the heart and lungs, routine measures which are considered of high value by all clinicians.

THE USE OF POSTERS AS A METHOD OF INSTRUCTION IN A DIABETIC CLINIC*

By John R. Williams, M.D., and Marion Vye, B.S., Rochester, N. Y.

THE value of personal instruction to diabetic patients is more and more realized by those who have to deal intensively with the problem. Indeed, it is our belief based on extended experience that the teaching of the patient is of equal importance with clinical and laboratory study of the disease, and that treatment will fail if those afflicted with diabetes do not fairly well understand the principles of dietary control, insulin therapy, and personal hygiene.

While most clinicians are in accord with these views, there is considerable variance in practice. Perhaps the most common is to give the patient one of the many well-known manuals on the market, and to supplement this with an occasional talk or demonstration by the dietitian. In our experience, textbooks are too technical, too difficult, and quite likely to be misunderstood, unless elucidated and interpreted by some one well versed in the subject.

The diabetic patient on admission to our clinic is given a loose-leaf book made up of mimeographed lessons or instructions. At his first class he is taught in simple language principles of food chemistry, metabolism, diabetes, and insulin action. The topics are presented in orderly manner, and the substance of what is said is printed and numbered in the lesson in his in-

[1]Private communication.

*Presented before the Hospital Dietetic Council at the Convention of the American Hospital Association, October, 1927.

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Fig. 1.—Group of colored posters made by cutting out and remounting pictures from well-known advertisements in current magazines. These are then given legends which have a special significance to the diabetic patient.

Fig. 2.—Practically every problem in the daily life of the diabetic patient is illustrated by these posters.

struction book. If he wishes to elaborate, he is encouraged to make notes on the blank page facing each lesson.

It has been our practice to teach by informal talks in the classroom and by demonstrations in the kitchen and laboratory. All this, of course, is very helpful and I presume is the method employed in every well organized clinic. It has its defects, however. Every diabetic problem cannot be elucidated by a laboratory demonstration, nor can abstract ideas always be set forth clearly by the spoken word. Diabetic patients gathered together in a classroom may represent all stages of education and mentality. Some may not understand the English language. Side by side may sit a high school teacher and an Italian laboring woman. The class may also include the extremes of age from the little child to the graybeard. The necessity for thorough instruction is not lessened by reason of these social and intellectual disparities.

It has been our experience from follow-up work that many patients do not grasp the significance of much class instruction. Moreover, when reliance is placed mainly in the spoken word as a medium for imparting information, but very few thoughts can be clearly expressed, and the efforts of the teacher are greatly circumscribed. Psychologists tell us that the eye will grasp an idea six times as readily as the ear, and that visual instruction is much more readily comprehended than oral. Professional advertisers have long since made use of this fact. Everyone knows that "there isn't a cough in a carload of Old Gold cigarettes," not from the spoken or written word, but from Briggs' cartoons. If you say Campbell's soup, immediately a beautiful picture of tomatoes, corn, celery, and so forth, dances before your eye. Everyone reads these advertisements and understands them.

It occurred to us, therefore, that the same pictorial method might be of use in the teaching of diabetic patients. We have cut from all sorts of magazines and catalogues colored pictures as well as cartoons, and by the liberal use of the paste pot and bristol board, we have made our own posters. Some of our friends in the canning industry have been kind enough to give us fruit and vegetable labels. In the original the author or advertiser may intend his picture to represent a particular idea which is defined in the accompanying text. We may use the picture to illustrate a wholly different thought. We may even employ it to condemn the product which is pictured (Figs. 1 and 2). We have not attempted to give publishers or advertisers credit for the use or misuse of their property. Ours is purely a humanitarian effort from which no possible harm to anyone can result.

Briefly, we have more than one hundred posters, each containing a striking picture. In preparing them, we endeavor to get pictures that are beautifully colored or humorous. We cut them out and mount them so that they look as nearly as possible like originals. We then have them neatly lettered with legends which will be brief and striking and yet will convey important messages. The finished posters are ten and one-half by thirteen and one-half inches. We have a linoleum bulletin board in our classroom which will hold twenty of these posters. When the series is complete it is our plan to have a different exhibit for each of the ten days, the average length of stay of pa-

tients in our clinic. In one day's lesson we endeavor to group all the common foods which are safe and useful in their order of comparative safety. In another group we show foods which are dangerous and forbidden. In other groups we illustrate ideas about exercise, bathing, tobacco, music, marriage, personal hygiene, the care of the feet, and so on. We do not rely entirely on this method of teaching but we use it merely as supplemental to class talks and demonstrations. Sometimes one poster will provoke a discussion among the patients and will stimulate interest and original thinking with them. We have found it helpful, in the training of little children, to adapt the same idea to scrapbook use. We have the child make such a book in which he pastes pictures of all the foods that are safe for him to use and those which he must avoid. We endeavor to see that he has a pictorial representation of every fact which is important for him to understand. We try thus to illustrate his daily life from the moment when he arises until he goes to bed at night. We give the child a set of can labels and encourage him to search through magazines for pictures which will be of service. Sometimes it becomes a family game, of more practical use and just as much fun as cross-word puzzling.

In conclusion, we present a poster method of instructing diabetic patients, which we use to supplement the older and well understood methods of teaching. In our hands it has been of value.

LABORATORY METHODS

A NEW ALDEHYDE REAGENT*

By Robert D. Barnard, S.B., Chicago, Illinois

THE rôle of acetaldehyde in intermediary carbohydrate metabolism[1] and in the fermentation of various sugars[2] makes it desirable to secure a reagent for the quantitative estimation of this substance in distillates from biologic materials. The reagent problem has been beset by many difficulties since there may be a concurrence of volatile substances which are closely allied to acetaldehyde chemically and may, therefore, introduce a considerable error. In this connection, attention is directed particularly to acetone which gives a positive Shiff reaction, forms a bisulphite addition compound, a cyanhydrin, a hydrazone, and an oxime just as does aldehyde. The method of Bougault and Gros[3] has been found by Chaikoff and Gee[4] to require an acetone correction factor. The ratio of acetone to aldehyde is in some instances greatly in favor of the former so it may readily be seen that correction for the amount of ketone present may be unsatisfactory.

It was felt that any improvement upon the reagents in use should embody the following points:

1. The reagent should be selective or specific.
2. It should be sensitive and allow estimation of small quantities.
3. The reaction should take place at room temperature and be fairly rapid to minimize volatilization and consequent loss of the aldehyde.

With these three points in mind the investigation (the results of which are herein reported) was undertaken. A reagent has been developed which appears to satisfy all the conditions imposed.

REAGENT

The reagent is a solution of acid fuchsin (Fuchsin S) decolorized by hydrazine hydroxide. It is prepared by adding 7.5 c.c. of 2 per cent acid fuchsin† to one liter of 0.042 per cent hydrazine hydroxide in water which had been boiled to reduce its carbon dioxide content. (The latter solution can be conveniently made up by diluting Eastman 42 per cent hydrazine hydroxide 1-1000.) Decolorization is complete at the end of two hours after which the reagent is ready for use. It has a faint brownish tint which is negligible in the Coleman Bell fuchsin but may be somewhat troublesome in some of the lots prepared by the National Anilin Works. The reagent has been kept for two months at ice box temperature without deterioration.

*From the Department of Physiology of the University of Chicago.
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†2.5 per cent where Coleman Bell acid fuchsin is used.

REACTION

After addition of aldehyde or acetone, in the cold, the reagent turns pink. It does not reproduce the exact shade of the original fuchsin but the color has an orange cast. The time elements are important for these two substances, the color developing gradually and reaching a maximum in the case of acetaldehyde in one to fifteen minutes, in the case of acetone in one to three hours, in both cases depending on the concentrations. Acetone must be present in great excess to give rise to the color within one hour and since the aldehyde reacts with such relative rapidity, it has been found possible to determine 0.0001 gm. of acetaldehyde in the presence of 0.1 gm. of acetone. The color due to the action of aldehyde upon the reagent fades within a few hours if the reaction mixture be exposed to air; when the reaction mixture is stoppered it has been observed to persist for at least a week. The color produced by acetone has been observed not to fade for at least a week, both in stoppered and unstoppered mixtures.

INTERFERING SUBSTANCES AND CONDITIONS

Free acids, when added to the reagent produce in it a color identical with pure acid fuchsin solutions. Their action is immediate which will distinguish them from aldehyde. The color produced by addition of acid, furthermore, is fully twice the depth obtained by either aldehyde or acetone and contains no element of orange.

Oxidizing agents such as molecular iodine, potassium ferricyanide, sodium persulphate, and potassium chromate and the salts of the heavy metals behave toward the reagent in a manner similar to free acids with the additional phenomenon of effervescence. In the cases of silver, copper, or mercury salts, the free metal is precipitated.

Bases or strong reducing agents will prevent the formation of the color by acetaldehyde and by acetone.

The color reaction with aldehyde will not take place in buffered solution.

METHOD OF APPLICATION

The distillate in which the quantity of aldehyde is to be determined should be unbuffered, neutral or alkaline to litmus and acid to phenolphthalein. One c.c. of the reagent is added to one c.c. of the distillate in the colorimeter cup and mixing effected by moving the plunger up and down through the solution three or four times. The color is compared at the end of fifteen minutes with a standard made simultaneously and in the same manner but using one c.c. of a stock solution of aldehyde containing 0.1 gram per liter.* These directions are for the Leitz micro colorimeter which will accommodate 2 c.c. of solution and the working range for the amounts of aldehyde and reagent given is between 0.02 mg. and 0.14 mg. For higher concentrations the distillate must be diluted to a point where the aldehyde content falls within this range.

*Since acetaldehyde upon standing often develops some free acid, which may interfere, it has been our custom to add one or two drops of 1 per cent sodium bicarbonate to the liter of stock solution.

DISCUSSION

As a colorimetric reagent, the one here presented offers some disadvantages. The color where small quantities of aldehyde are involved may be very faint and entirely unsuited for reading. The bi-color nature of the reaction mixture has made it impossible to use a permanent standard. The range has narrow limits.

The reagent is sensitive to one part of aldehyde in one hundred thousand; the reaction takes place at room temperatures and a determination may be effected in fifteen minutes.

SUMMARY

A new quantitative reagent for acetaldehyde in the presence of an excess of acetone is described.

The author extends his heartiest thanks to Mr. R. D. Templeton and Dr. A. J. Carlson for their cooperation in ascertaining the quantitative usefulness of the reagent.

REFERENCES

[1]Neuberg, C.: Ber. Chem. Ges., 1922, lvi, B, 3624.
[2]Peterson, W. H., and Fred, E. B.: Jour. Biol. Chem., 1920, xliv, 29.
[3]Bougault, J., and Gros, R.: Jour. Pharmacol. et chem., 1922, vii, 265.
[4]Gee, A. H., and Chaikoff, I. L.: Jour. Biol. Chem., 1926, lxx, 151.

DOUCHE AND SHOWER AND SPECIAL CABINET FOR GASTRO-INTESTINAL TUBES*

By Moses Einhorn, M.D., New York

THE recent rapid increase in the use of Ewald and duodenal tubes for obtaining gastric content after Ewald or fractional test meals and for the performance of biliary drainage has created the need for an apparatus which would solve the problem of the cleansing and storing of the tubes used in these operations. The importance of using absolutely clean tubes in order to obtain the best results cannot be overemphasized. The technician must be sure that the insides of the tubes which he uses are free from any remnants of previous examinations, otherwise the results he obtains will be open to question, since the particles remaining from previous examinations will influence the results obtained in later tests. Secondly, since it is impossible to use a new tube for each patient, it is necessary to take every precaution to prevent these tubes from being the agencies for the spread of contagious diseases by administering a thorough cleansing before and after each examination. The length of these tubes together with other intrinsic difficulties, particularly in the case of the narrow duodenal tubes, has made the problem of cleaning and storing them a difficult one, and it is in the hope that my colleagues will find my

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apparatus as convenient and efficient a solution of the problem as I have found it to be that I present the apparatus described below.

Two extensions, each of which contains a stopcock, are attached respectively to the back of the hot and cold water faucets of a sink. A union connects these extensions and from the middle of this union an arm projects. To this projection, the actual apparatus is attached (see Fig. 1). The apparatus consists of a long tube on the bottom of which three specially devised faucets are attached, four inches apart. These faucets terminate in a disk the center of which contains an outlet into which the standard hard rubber Ewald connection fits exactly. The remainder of the tube is perforated in such fashion

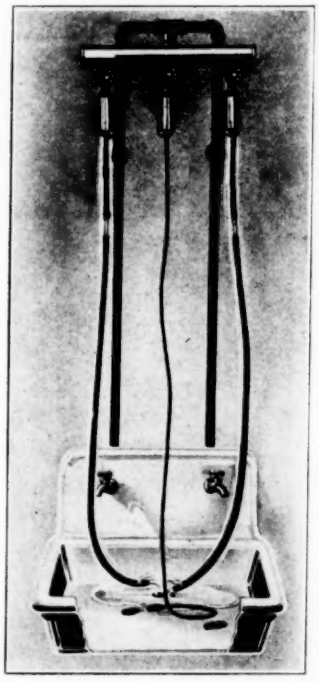

Fig. 1.

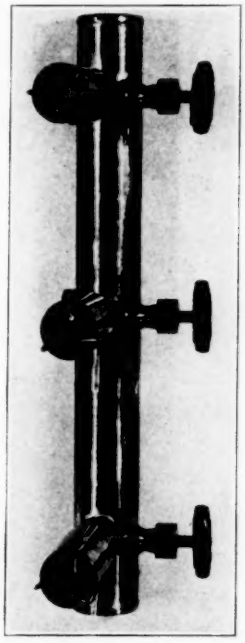

Fig. 2.

that the shower of water emerging therefrom strikes against and completely envelopes the outside of the attached tube. A clip has been devised which permits of the insertion or withdrawal of the tube with a single motion of but one hand (see Fig. 2). The reverse end of a standard hard rubber Ewald connection is narrowed so that it fits into a duodenal tube. By the use of this slightly altered connection piece, this apparatus cleans duodenal tubes in the same fashion as Ewald tubes. The number of outlets may be increased when a greater number of tubes are to be cleaned.

One of the most attractive features of this apparatus is the extreme simplicity of its operation since it requires no attention or effort upon the part of the technician. The sink faucets are closed and the stopcocks in the extension are opened according to whether the tubes are to be cleaned with

hot, lukewarm, or cold water. The tubes are then inserted in the special faucets. The water will now flow into the central tube of the apparatus and by opening the stopcocks on this tube one or all of the tubes may be cleaned. Once the water is turned on and the tubes are inserted, the technician is free to do other work until he is satisfied that the tubes have been thoroughly cleaned.

A special cabinet has been constructed for the storing and drying of these tubes (see Fig. 3). A bar across the top of this cabinet is equipped with clips of the type previously described. Since the cabinet is almost five feet high,

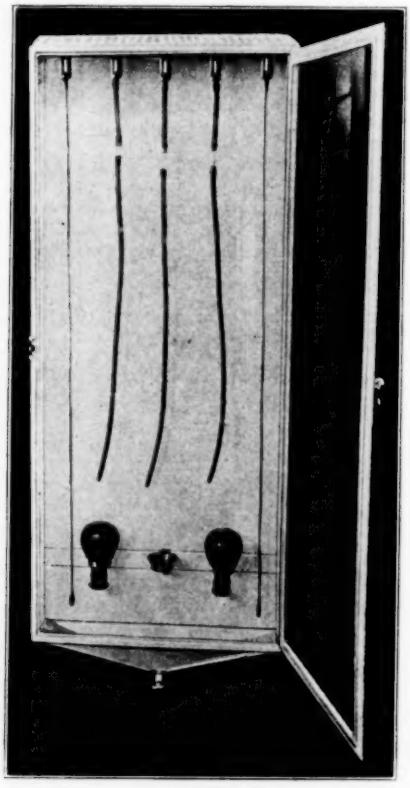

Fig. 3.

the tubes are able to hang vertically when they are inserted. The Ewald bulbs may be stored in holders which are provided for that purpose. The floor of the cabinet is made in such fashion as to permit drainage of the accumulating moisture into a central outlet which is regulated by a stopcock. Numerous perforations in the sides and top of the cabinet supply a rapid circulation of air which causes the tubes to be dried quickly. The cabinet is provided with a glass-paned door which, when shut, acts to prevent the entrance of dust.

I have observed that the following advantages are obtained by using the above described apparatus:

1. It is now possible to clean the tubes with water of any temperature.

2. The tubes are now thoroughly cleaned both on the inside and outside.

3. The technician can save time by cleaning many tubes in a single operation instead of cleaning each one separately as was formerly necessary.

4. Except for the insertion of the tubes and the turning on of the water, the above described method of cleaning the tubes requires no attention or effort on the part of the technician.

5. The tubes are more thoroughly cleaned than previously since the water descends from a great height and consequently has sufficient power to remove all the particles from the inside and outside walls of the tubes.

6. Since the tubes hang vertically in the cabinet, folding or misshaping of the tubes is prevented.

7. The cabinet enables the tubes to be kept clean and sanitary.

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THE CLINICAL VALUE OF THE SPINAL FLUID TEST OF TAKATA AND ARA*

By B. L. Monias, Ph.D., Chicago

THE difficulties encountered sometimes in preparing a satisfactory colloidal gold solution for the Lange's test have led to the device of more simple reactions which can be performed also in small laboratories or in the physician's office. None of these reactions, however, has so far been able to replace the Lange's test. Recently Takata and Ara[1] have reported on good results with a simple reaction which is based on the following principle: By mixing a mercuric chloride solution with sodium carbonate in the presence of the normal spinal fluid a colloidal solution of mercuric oxide is formed which turns a deep bluish-violet by adding a solution of diamond-fuchsin. With pathologic spinal fluids the reaction is different. The abnormal protein content of the latter causes either a flocculation of the mercuric oxide with a discoloration of the fluid or color changes from bluish-violet to pink with practically no precipitation. The first type of reaction, according to Takata and Ara, is typical of syphilis of the central nervous system while the change of the color occurs in bacterial meningitis. These observations have been confirmed during the last year by several German investigators, notably Blum,[2] Jacobsthal and Joel,[3] Haitsch,[4] Knigge,[5] and Nicole.[6]

I have used the Takata Ara test in connection with the other spinal fluid reactions on over one hundred spinal fluids and since the results have been very satisfactory I feel justified in making the American laboratory workers familiar with it.

*From the Uihlein Memorial Laboratory of the Grant Hospital of Chicago.
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THE TECHNIC OF THE REACTION

The following solutions are required:

1. A 0.3 per cent solution of sodium chloride.

2. A 10 per cent solution of sodium carbonate. This solution is recommended by Takata and Ara but can be replaced by a N/10 sodium hydroxide solution of which the double amount is taken. In cases of meningitis the color change is more distinct with the sodium hydroxide than with the carbonate.

3. A 0.5 per cent solution of mercuric chloride.

4. A 0.02 per cent solution of diamond fuchsin in distilled water.

The spinal fluid must be free from blood and should not be kept longer than twenty-four hours before making the test. The test: A series of 10 standard Wassermann test tubes are set up and one c.c. of the 0.3 per cent sodium chloride solution is placed in the second tube and up to the tenth tube. One c.c. of the spinal fluid is placed in the first and second tubes and after mixing, one c.c. is withdrawn from the second tube and filled in the third tube, and this procedure is repeated with the following eight tubes. The last c.c. of the dilution is discarded. There is now a range in the dilution from the concentrated spinal fluid in the first tube to 1:512 in the last tube. One drop from a capillary pipette of the sodium carbonate or two drops of the sodium hydroxide solution are added to each tube and mixed well. The test is completed by the addition of the Takata Ara reagent. This reagent consists of equal parts of the mercuric chloride and fuchsin solutions mixed immediately before using. The solutions of mercuric chloride and fuchsin keep indefinitely. Three-tenths c.c. are used in each tube. The test tubes are shaken thoroughly and kept at room temperature. Takata and Ara first recommended three readings, namely, after fifteen minutes, thirty minutes, and twenty-four hours. In a later publication Takata said that only one reading should be taken after twenty-four hours. From my experience I would say that some information can be obtained after thirty minutes and that the result becomes final after twelve hours.

Two different forms of reactions may take place with pathologic spinal fluids. First, the mercuric oxide flocculates, absorbing the dye. The strongest reaction causes a complete discoloration of the fluid and large, deep blue precipitates settle at the bottom of the tube. If the reaction is less complete, the supernatant fluid remains more or less blue and the sediment is finer. The intensity of the flocculation is recorded in the following way: (+), +, $+,^{1}$ $+,^{2}$ $+.^{3}$

Second, the color of the solution changes from bluish-violet to reddish-violet, purple or pink. The fluid remains clear or becomes cloudy. This result is indicated by a circle: O reddish-violet, O^{1} purple, O^{2} pink. If precipitation and color change occur simultaneously the two signs are combined, e.g., O^{1+2} indicating a marked precipitation with a change of the color to purple.

RESULTS

A. *Normal Spinal Fluids.*—Five fluids have been selected to demonstrate the reaction. The solution has a deep bluish-violet color and remains so. There is sometimes a slight precipitation in the last three tubes which is of no significance. I suggest, therefore, that the last three tubes with the highest dilutions be omitted.

TABLE I

NORMAL SPINAL FLUIDS

NO.	WASSERMANN	KAHN	LANGE	NONNE	TAKATA ARA
1	neg.	neg.	neg.	neg.	– – – – – – – – – –
2	neg.	neg.	neg.	neg.	– – – – – – – – – (+)
3	neg.	neg.	neg.	neg.	– – – – – – – + + +
4	neg.	neg.	neg.	neg.	– – – – – – – – – –
5	neg.	neg.	neg.	neg.	– – – – – – – (+) + +

TABLE II

B. SYPHILITIC SPINAL FLUIDS

NO.	CLINICAL DIAGNOSIS	WASSERMANN BLOOD	WASSERMANN SPINAL FLUID	KAHN BLOOD	KAHN SPINAL FLUID	NONNE	LANGE	TAKATA ARA
1	General paresis	+ + + +	+ + + +	+ + + +	+ + + +	+	355543200000	$+^{3}+^{2}+^{1}+$ (+) – – – – –
2	" "	+ + +	+ + + +	+ + +	+ + + +	+ +	555520000000	$+^{3}+^{3}-$ – – – – – – –
3	" "	+ +	+ + +	+ +	+ + +	+ + +	555531000000	$+^{3}+^{3}+^{1}-$ – – – – – –
4	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	210000000000	$+^{2}-$ – – – – – – – – –
5	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	421000000000	$+^{3}-$ – – – – – – – – –
6	" "	+ + + +	+ + + +	+ +	+ + +	+ + +	554444200000	$+^{3}+^{3}+^{3}+^{1}-$ – – – – –
7	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	555554200000	$+^{3}+^{3}+^{3}+^{3}+^{3}+^{2}+^{2}-$ – –
8	" "	+ +	+ + +	+ + +	+ + +	+ +	555553100000	$(O^{+2})^{1}+^{1}-$ – – – – – – –
9	" "	+ +	+ +	+ +	+ +	+	354431000000	$+^{3}+^{3}-$ – – – – – – –
10	" "	+ + + +	+ + + +	+ + + +	+ + + +	+	555443210000	$+^{3}+^{1}+^{1}-$ – – – – – –
11	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	554333210000	$+^{3}+^{2}+^{1}-$ – – – – – –
12	" "	+ +	+ +	+ + + +	+ + + +	+ + +	554443210000	$+^{3}+^{3}-$ – – – – – – –
13	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	555443210000	$+^{3}+^{1}-$ – – – – – – –
14	" "	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	555554433210	$(O^{+3})^{1}+^{3}+^{3}+^{3}+^{3}+^{3}-$ – – –
15	Tabes dorsalis	+	+	+ +	+	+	123321100000	$+^{1}+(+)-$ – – – – – –
16	" "	neg.	+	neg.	+	+	001221000000	$+(+)-$ – – – – – – – –
17	" "	+ +	+ + + +	+ +	+ + +	±	112211000000	– – – – – – – – – –
18	Syphilis of CNS.	+ + + +	+ + + +	+ + + +	+ + + +	+ +	224555530000	$+^{3}+^{2}+^{1}+(+)-$ – – – –
19	" "	+ + +	+ +	+ + +	+ +	+	112332100000	$+^{3}+^{3}-$ – – – – – – –
20	" "	+	+ +	+ +	+	±	223321000000	$+^{2}+^{1}-$ – – – – – – –

The strongest reactions are obtained in general paresis. Complete flocculation takes place constantly in the first tube and also often in the second, third and sometimes in the fourth and fifth tubes. The precipitate is stained a deep blue. In two instances the color in the first tube had changed to purple.

In syphilis of the central nervous system the reaction is similar to that in general paresis. A differentiation between a paretic and a syphilitic curve is not possible. In tabes dorsalis the reaction is less distinct or negative.

All of the eight cases showed a very distinct change of the color to pink in the first tube. The other tubes remained almost unaffected with the tuberculous spinal fluid, while in epidemic meningitis, the content of the second

TABLE III

C. MENINGITIS

NO.	CLINICAL DIAGNOSIS	NONNE	LANGE	TAKATA ARA
1	Tuberculous meningitis	++++	000011321000	O^2 O^1 --------
2	" "	+	000111000000	O - --------
3	" "	++++	000123100000	O^2 O --------
4	Streptococci meningitis	++	001222210000	O^2 - --------
5	Epidemic meningitis	++++	000022110000	O^2 O^2 --------
6	" "	++++	000012210000	O^2 O^2 --------
7	" "	+++	000011100000	O^2 O^2 O -------
8	" "	+++	000000111100	O^2 O^1 O -------

and sometimes also of the third tube assumed a pink coloration. In none of these cases was there any precipitation, although the fluid in two instances appeared slightly cloudy.

SUMMARY

The Takata Ara test is based upon the formation in normal spinal fluids of colloidal mercuric oxide when mercuric chloride and sodium carbonate or sodium hydroxide are added. Fuchsin serves as an indicator and stains the fluid a bluish violet.

Pathologic spinal fluids either produce a precipitation of the mercury with absorption of the dye or a change of the color to purple or pink.

The flocculation is characteristic of syphilis while the change of the color is observed in bacterial meningitis.

The test has given satisfactory results on over one hundred spinal fluids and compares favorably with the Lange's colloidal gold reaction.

In tabes dorsalis the test is less sensitive than the Lange's reaction.

The test does not call for special technical skill and for special glasswares and triple distilled water. It can easily be performed in small laboratories or in the office.

REFERENCES

[1]Takata, M., and Ara, K.: A new colloidal liquor reaction and its practical results, Tokyo, 1926 (in German).
[2]Blum, E.: Ztschr. f. d. ges. Neurol. u. Psychiat., 1927, cx, 504.
[3]Jacobsthal, E., and Joel, M.: Klin. Wchnschr., 1927, vi, 1896.
[4]Haitsch, E.: Psychiat.-Neurol. Wchnschr., 1927, xxix, 3.
[5]Knigge, F.: München. med. Wchnschr., 1926, lxxiii, 1836.
[6]Nicole, R.: Ztschr. f. Klin. Med., 1927, cvi, 192.

"A CRITIQUE OF THE LIPASE 'PICTURE' METHOD"*

By K. George Falk, Helen Miller Noyes, and Kanematsu Sugiura,
New York

IN A PAPER with the above title,[1] Harry Beckman, M.D., expresses disappointment with the method of determining and presenting certain ester-hydrolyzing actions by us.[2]

The disappointment of Beckman is stated to be based upon three sets of objections. Work on rat spleen extracts only is quoted.

1. Serum lipase is not taken into account in the method. No experimental evidence is presented by Beckman as to the amount of serum present in the material or of the relative lipase activities of the serum and tissue extracts. The criticism is based upon the finding of a serum lipase by Rona and Michaelis in 1911 and by Rona and Bien in 1914, and upon the different colors of ten splenic extracts prepared by Beckman. It may be added that in some unpublished experiments carried out in the Harriman Research Laboratory, it was found that the action on benzyl acetate of rat serum was between 50 and 75 per cent of the action of phenyl acetate, while with the rat spleen extract it was less than 14 per cent. This in itself would show that the serum, if present in the spleen, exerted a negligible influence.

2. Whether the rats are killed by a crushing blow on the head or by ether inhalation is stated to show different results with the spleen extracts. The method of testing the enzyme actions of Falk, Noyes, and Sugiura was not used by Beckman, but a "modification to meet experimental conditions" was developed. The extracts used by Beckman were very much more dilute than those used in the original method. Beckman in Table I of his paper gives ten results on extracts of spleens of individual rats on methyl butyrate. In the first place, using N/100 NaOH gives numbers comparable in magnitude to those found in the original method where N/10 NaOH was used, but the actions when translated into the latter terms ranged in the extreme cases from 0.04 to 0.55 c.c. Nothing is said as to whether duplicate determinations were made. The extracts were stated to be "neutralized" before incubation with the esters, but whether indicator was added to the solutions and was present throughout the tests and whether a definite P_H was attained is not stated. If the actions on methyl butyrate were the largest found, obviously the extent of the ester hydrolyses in general was rather small upon which to base final conclusions. It may also be asked, why results on rat spleen only were presented by Beckman, and detailed results (in Table I and Fig. 2) on methyl butyrate alone, to show the irregular actions, when it was specifically stated on page 203, line 2, of the paper by Falk, Noyes, and

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Sugiura, in connection with the rat spleen results that "the methyl butyrate actions were somewhat irregular."

However, Beckman plots his experimental results on six esters (including benzyl benzoate which was not used in the original method), and then states:* *"It will be seen that there is a certain characteristic 'picture' for all the animals in the group killed by a blow,* but that very considerable quantitative differences exist between individuals of the group. *In the group killed by ether the same type of 'picture' is again seen,* but the quantitative differences between individuals are even greater than in the blow group." In view of the inaccuracies introduced by Beckman in his modification of the method, his conclusion agrees remarkably with the conclusions given in the original paper. There it was stated in the Discussion, page 211: "The results which have been presented show definite characteristic 'pictures' for the ester-hydrolyzing actions of the rat tumor and tissues. . . . The relative actions of the various enzyme materials proved to be of the most significance in the interpretation of the results, but the absolute actions should also be considered." Also, on page 188: "In some cases the amounts of actions for the same concentration of a given tissue in different series differed considerably." Furthermore, a study of the curves given by Beckman in which the absolute actions, not the relative actions, were plotted, shows considerable variation in each set, and overlapping of the two sets. The two sets of curves, one obtained with rats killed by a blow, the other with rats killed by ether, are not distinct. Except for three results with one ester, methyl butyrate, for which the results are admittedly variable, it would not be possible to state definitely to which set a given curve belonged.

Evidently Beckman has contributed nothing, either constructive or destructive, in his experimental study.

3. The method is stated not to take into account the escape of esters from contact with the extract. The only experiment which Beckman gives in this connection (aside from indicating boiling points in a graph) is as follows: "Exposing equal surfaces of the substances in calibrated tubes in the incubators for a given time will show the quite different rates at which they disappear." In the original method 0.2 to 0.5 c.c. of esters were present in each case with 15 c.c. of extract or mixture in a stoppered 60 c.c. Erlenmeyer flask. It is difficult to see how Beckman's experiment with pure esters applies to the method. He presents no evidence that esters evaporate either at different rates or at all under the conditions of the experiments.

CONCLUSION

The objections expressed in the "Critique" of Beckman are unfounded.

REFERENCES

[1]Beckman, H.: JOUR. LAB. AND CLIN. MED., 1927, xiii, 214.
[2]Falk, K. G., Noyes, H. M., and Sugiura, K.: Jour. Biol. Chem., 1924, lix, 183.

*Italics were not used by Beckman.

CONTROL TESTS OF A HALDANE CHAMBER APPARATUS IN THE METABOLIC STUDY OF ADULT ALCOHOLIZED POULTRY*

By Thorne M. Carpenter, Ph.D., Boston, Mass.

IN CONNECTION with a study begun in 1918 of the metabolism of poultry as affected by the inhalation of the vapor of ethyl alcohol, a number of gaseous exchange measurements were made upon several adult hens, using the Haldane (1892) chamber method for animals. Occasionally results were obtained which were distinctly aberrant, and it was thought advisable to study the difficulties of this method by running control tests with burning ethyl alcohol.

The general principle of the method is as follows: An air-tight chamber containing the animal is ventilated by a current of air free from carbon dioxide and water vapor; the carbon dioxide and water vapor in the air leaving the chamber are retained by suitable absorbers which are weighed periodically. The chamber containing the animal is similarly weighed and the oxygen absorbed by the animal is calculated by subtracting the loss in weight of the chamber and contents from the sum of carbon dioxide and water vapor.

In the decade following its description by Haldane, the method was used by Pembrey and coworkers for mice (Pembrey, 1894), rabbits (Pembrey and Gürber, 1894, Mutch and Pembrey, 1911, Kennaway and Pembrey, 1912), eggs and chicks (Pembrey, Gordon and Warren, 1894), marmots, dormice, and hedgehog (Pembrey, 1901, 1903), and rats (Pembrey and Spriggs, 1904). During the last decade it has been more widely applied both biologically and geographically. Since Danoff (1919) used it in the study of the metabolism of rats, it has been employed in the Institute of Physiology at Berne in an extensive series of studies with small animals. The method has been applied by others to the metabolism studies on rats (Fridericia, 1913, Cramer and McCall, 1917, Nakayamo, 1924, Reiss and Weiss, 1925, Harris, 1925, Mitchell and Carman, 1926, and Lee, 1927), rabbits (Marine and Lenhart, 1920, Bertschi, 1920, Isenschmid, 1920, Dittler, 1922, Také, 1922, and Okuma, 1926), dogs (Hédon, 1921, 1922, 1926), and chickens (Mitchell and Haines, 1927 *a*, 1927 *b*). Terroine and coworkers (Terroine and Trautman, 1926, 1927) have used it on a variety of small animals. Marine (1922) gave a detailed description of the apparatus as applied to infants, rabbits, and cats, and Hédon (1926) described the apparatus as applied to dogs and discussed the magnitude of the errors theoretically possible. Boothby and Sandiford (1924) comment that "as the oxygen is indirectly determined * * * the respiratory quotient may be considerably disturbed by the fact that a large error may result from the indirect method of oxygen determination, on account of the

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difficulty of accurately measuring the water vapor." Krogh (1916) stated that "the Haldane apparatus, which allows the determination both of carbon dioxide and oxygen is extremely accurate."

Many of the users of this method state that blanks have been run, tests for tightness made, and efficiency tests of the absorptive power of the water and carbon dioxide retainers have been conducted, but very few have published any numerical results. Some have taken the low respiratory quotients found with fasting animals as an indication that the method was correct. Haldane (1892) and Pembrey and Gürber (1894) reported quantitative results for the recovery of known amounts of water and carbon dioxide, Hauri (1919) determined the error in the water determination, and Isenschmid (1920) standardized his apparatus with known amounts of carbon dioxide developed by the addition of hydrochloric acid to calcium carbonate. It is recommended that investigators give at least a quantitative summary of blank tests and controls of efficiency of absorption.

A control of the Haldane chamber apparatus in experiments on animals was made by Fridericia (1913) in which he used a closed circuit system both as a Regnault-Reiset apparatus and as a Haldane apparatus. The oxygen absorption was determined from the contraction in volume of a gasometer connected to the circulating closed system, and the water vapor and carbon dioxide output were determined by the gains in weight of absorbers containing sulphuric acid and soda lime. At the end of each period the chamber was detached and weighed so that the oxygen absorption could be calculated indirectly as well as directly determined. Rats were used in 13 experiments from two and one-half to twenty-four hours in duration and the agreement between the two methods of oxygen determined averaged inside of 0.5 per cent, when the length of the experiment was more than three and one-half hours.

Cori and Cori (1926) have made a similar comparison on rats in 40 experiments, of the oxygen determined by means of the closed circuit with that obtained by the Haldane method, and report an average agreement of 1.4 per cent for three to four hour periods. The average difference in the respiratory quotients was 0.01. In a recent paper (Cori and Cori, 1928) they confirm their earlier results.

CONTROL TESTS WITH BURNING ETHYL ALCOHOL

Apparatus and Technic.—The chamber used in the series reported here was made of sheet copper and was 37 cm. long, 23 cm. wide, and 28 cm. high with a volume of about 25 liters. An opening at the top made of brass angle was 21 cm. long and 16.5 cm. wide. This is large enough to permit the entrance of a 2 to 3 kg. fowl. A pane of plate glass was sealed in with physicists' wax to cover the opening. At one end of the bottom of the chamber two steel pivots were fastened on the outside which rested in sockets on a wooden frame. The other end of the bottom rested on two metal springs. At the top of the chamber above the end which rested on springs, a pointer of sheet metal was attached. With this arrangement, the movements of an animal inside of the chamber were registered upon the smoked paper on the moving drum

of a kymograph. The amplitude of the record depended upon the length of the pointer and the sensitivity depended upon the tension of the springs on which one end of the chamber rested. The ventilating air entered at a corner in the lower part of the chamber wall and went out at the top at a diagonally opposite point. On the end where the ventilating air left, a thermometer was inserted in a tubular enlargement in order to obtain the temperature of the air in the chamber.

Absorber Systems.—The ventilating air from the room or from out of doors passed to the chamber through a calcium chloride tower filled with soda lime and through two Drechsel gas washing bottles containing concentrated sulphuric acid. From the chamber it then passed through two Drechsel bottles containing sulphuric acid, a calcium chloride tower filled with soda lime, two Drechsel bottles containing sulphuric acid, a bottle containing a solution of barium hydroxide, a 10 liter Bohr gas meter and a small rotary blower. The purpose of the barium hydroxide container was to make sure that the carbon dioxide was completely absorbed from the air coming from the chamber. If the second of each pair of water absorbers was weighed, a control was maintained of the efficiency of the water absorption. So much trouble developed from time to time with cracks appearing in the wax seal of the opening of the chamber, that it was found advisable to test the completeness of absence of leaks at the end of each period by putting suction on the chamber and absorber system equivalent to more than that obtained under running conditions. After the chamber was weighed, it was connected to the absorber train following it, and suction was applied to this system, the ingoing tube of the chamber being closed, and the manometer placed at the exit of the last bottle of the absorber train. In most cases, however, the absorber train was tested while the chamber was being weighed, and after weighing, the chamber was tested alone. The leaks in the chamber were usually due to the contraction of the wax away from the metal. This procedure was recommended by Haldane (1892) and its use in our experiments with poultry has proved valuable in detecting leaks in the system which might have passed otherwise unnoticed. It is to be strongly recommended as an essential part of the routine with the Haldane chamber method.

Alcohol Lamp and Technic of a Control Test.—The lamp used was an Erlenmeyer flask or bottle of 100 c.c. capacity into which was inserted a rubber stopper with a wick (cotton string) running through glass tubing placed in a hole of the stopper. The bottle was partially filled with ethyl alcohol of known density, lighted, and its rate of burning determined by weighing on a balance at intervals, and adjustment of the rate (1 to 2 gm. per hour) was made by raising or lowering the wick. The lighted lamp was then placed in the chamber and the latter was then connected to the ventilating air current which varied from 4 to 9.5 liters per minute in these experiments. The opening of the chamber was then sealed and the ventilation continued for about one-half hour. The chamber was then closed off from the ventilating air current by screw pinch cocks on rubber tubing connected to the ends, and was weighed as quickly as possible. A Sauter balance with a sensitiv-

ity of one centigram under a load of 5 kg. was used for all the weighings. As soon as possible after the weighing the chamber was connected to the ventilating system and the air current started through a previously weighed set of absorbers for water vapor and carbon dioxide. Occasionally too much time was taken for weighing the chamber and the flame went out, but when care was taken to place beforehand on the balance pan the estimated weight of the chamber, little difficulty was experienced in this regard.

RESULTS OF ALCOHOL CHECK EXPERIMENTS

The results of 8 experiments are shown in Table I. In these experiments it was assumed that the losses in weight from the chamber represented the weights of alcohol which were burned in the individual periods. This assumption would mean that there was no change in weight of the chamber other than that due to the loss due to the burning of alcohol, i.e., no extra water was vaporized and the composition of the residual air remained unchanged. The periods are for the most part of thirty minutes' duration or more, but the weight of alcohol burned is of more significance than the length of the periods. The alcohol burned was usually over 1 gram, but in Experiment 8 it was 0.43 gram. In all experiments, except No. 7, there was a decrease in the amount of alcohol burned in each succeeding period. This is the usual experience with alcohol burning in a lamp such as was used in these experiments. The percentage of water recovery is on the whole higher than theory, which would indicate that the loss in weight of the chamber included some vaporized water other than that which came from the alcohol. The weights of water were for the most part over 1 gram, with the exception of periods in Experiments 2 and 8. The carbon dioxide on the whole averages slightly lower than theory, indicating that there was less alcohol burned than computed from the loss in weight of the chamber. The weights of carbon dioxide absorbed in the individual periods are somewhat higher than those for the water, for the most part over 2 grams. The oxygen recovery on the whole is slightly better than that for carbon dioxide and the quantities in the various periods are slightly higher than those for carbon dioxide. The quantities of water vaporized, carbon dioxide produced, and oxygen used, are for the most part not much larger than those involved in the metabolism of adult hens. For 132 periods in 25 experiments with hens in which the duration of the periods averaged fifty-three minutes, the values were: water vaporized, 1.75 grams; carbon dioxide eliminated, 1.79 grams; and oxygen absorbed, 1.61 grams. These alcohol check tests form a suitable basis for estimating the accuracy of results in studies with adult poultry.

The most important calculation in the table is the respiratory quotient in the different experiments. Experiment 5 was the best in individual periods, but for the most part the average of each experiment comes within 0.02 of the theoretical respiratory quotient for alcohol. It is evident that with extreme carefulness, respiratory quotients may be obtained for individual periods with this method, which are very satisfactory as shown by Experiment 5 and the two periods of Experiment 2. As the respiratory quotients for the experiments as a whole are satisfactory, the indication is that the

TABLE I

RESULTS OF ALCOHOL CHECK EXPERIMENTS WITH A HALDANE CHAMBER RESPIRATION APPARATUS

EXPERIMENT NUMBER AND PER CENT OF ALCOHOL	DURATION OF PERIOD	LOSS FROM CHAMBER (ALCOHOL)	WATER		CARBON DIOXIDE		OXYGEN		RESPIRATORY QUOTIENT
			FOUND	PER CENT OF THEORY	FOUND	PER CENT OF THEORY	FOUND	PER CENT OF THEORY	
	HR. MIN.	GM.	GM.		GM.		GM.		
	46	0.98	1.22	108.0	1.77	103.5	2.01	107.5	0.640
1	46	0.97	1.12	100.0	1.57	92.9	1.72	93.0	0.664
91.3 per cent	46	0.97	1.16	103.6	1.60	94.7	1.79	96.8	0.650
	46	0.83	1.03	107.3	1.45	100.0	1.65	104.4	0.639
Total	3 4	3.75	4.53	104.1	6.39	97.7	7.17	100.4	0.647
2	45	0.71	0.83	101.2	1.30	104.8	1.42	105.2	0.666
91.3 per cent	45	0.56	0.62	95.4	1.01	103.1	1.07	100.0	0.686
Total	1 30	1.27	1.45	98.6	2.31	104.1	2.49	102.9	0.678
	60	1.82	2.16	102.4	2.92	91.8	3.26	94.2	0.651
3	62	1.70	1.94	98.5	2.85	96.0	3.09	95.4	0.671
91.3 per cent	60	1.52	1.92	109.1	2.80	105.7	3.20	110.7	0.637
	60	1.51	1.87	106.9	2.77	105.3	3.13	109.1	0.644
	60	1.40	1.58	97.5	2.35	96.3	2.53	95.1	0.675
Total	5 2	7.95	9.47	102.8	13.69	98.7	15.21	100.5	0.654
	62	1.31	1.45	96.7	2.05	96.7	2.19	94.8	0.681
4	60	1.17	1.37	102.2	1.78	94.2	1.98	96.1	0.654
84.5 per cent	60	1.10	1.33	105.6	1.62	91.0	1.85	95.4	0.637
	60	1.02	1.27	108.5	1.56	94.5	1.81	100.6	0.627
Total	4 2	4.60	5.42	102.7	7.01	94.3	7.83	96.7	0.651
	60	2.01	2.35	101.7	3.06	94.2	3.40	96.0	0.655
5	60	1.67	1.97	102.6	2.73	101.1	3.03	103.1	0.655
84.5 per cent	60	1.50	1.78	103.5	2.55	105.4	2.83	107.2	0.655
	60	1.54	1.78	100.6	2.53	101.6	2.77	102.2	0.664
	60	1.49	1.71	100.0	2.34	97.1	2.56	97.3	0.665
Total	5 0	8.21	9.59	101.8	13.21	99.6	14.59	100.9	0.659
6	30	1.24	1.48	104.2	1.89	94.5	2.13	97.7	0.645
84.5 per cent	30	1.07	1.22	99.2	1.73	100.0	1.88	99.5	0.670
Total	1 0	2.31	2.70	101.9	3.62	97.1	4.01	98.5	0.655
	63	1.18	1.43	105.9	2.01	105.2	2.26	108.7	0.647
7	64	1.30	1.51	101.3	2.09	99.5	2.30	100.4	0.661
84.5 per cent	64	1.28	1.45	98.6	2.03	98.1	2.20	97.3	0.672
	64	1.25	1.41	98.6	1.94	96.0	2.10	95.5	0.672
Total	4 15	5.01	5.80	100.9	8.07	99.8	8.86	100.3	0.665
	45	1.18	1.32	97.8	1.78	93.2	1.92	92.3	0.674
8	45	1.19	1.36	100.0	1.91	99.5	2.08	99.0	0.668
84.5 per cent	25	0.43	0.46	93.9	0.68	98.6	0.71	93.4	0.697
Total	1 55	2.80	3.14	97.8	4.37	96.7	4.71	95.5	0.673
Average of periods	--	1.24	1.45	101.8	2.02	98.4	2.24	99.6	0.659
Average deviation of periods	--	0.27	0.32	3.2	0.47	3.8	0.52	4.4	0.013
Mean deviation of periods	--	0.36	0.43	4.0	0.59	4.5	0.66	5.3	0.016

difficulty of obtaining theoretical results for carbon dioxide and water in the individual periods is not due to the variation in the quantities themselves but due to the difficulty of measuring accurately the amount of alcohol burned. If there were appreciable errors in any one of the three factors measured, the result would be an error in the fourth factor which is calculated, namely, oxygen, and an error in the ratio between carbon dioxide and oxygen, that is, the respiratory quotient.

The average deviation and the mean deviation of the periods are given at the bottom of the table. For the quantities measured these two constants show the degree of variability of the amounts of the weights of losses of the chamber, water, and carbon dioxide. On the other hand the constants for the percentages show the variability in the accuracy with which the observations were made. In general the average deviation of the percentages is between 3 and 4.5, while the mean deviation of the percentages is between 4 and 5.5. Out of a total of 29 periods, sixteen of the periods for the water, fifteen of the periods for carbon dioxide, nineteen of the periods for oxygen, and twenty of the periods for the respiratory quotient are within the mean deviation. As the mean deviation is less percentagewise for the respiratory quotient than for any of the other three factors, it is easier to obtain quotients in alcohol checks than it is to measure water, carbon dioxide, and oxygen. The reason for this is that the respiratory quotient depends upon the accuracy of the weighings of the individual parts, while the percentage recovery of oxygen, carbon dioxide, and water depends upon whether the loss in weight of the chamber represents solely the alcohol burned.

As there was no source of carbon dioxide other than the burning alcohol, and the completeness of absorption was controlled by the barium hydroxide solution, the weight of carbon dioxide obtained was used as the basis of calculating the amount of alcohol burned and the percentages of recovery for oxygen and water were computed. The average percentages for water and oxygen were 101.5 and 101.1 respectively, so that on the whole the results are not much changed for the average of the individual experiments on the assumption that the carbon dioxide was an indication of the alcohol burned. The calculation of the average deviation and the mean deviation for the individual periods when carbon dioxide is assumed as the measurement of the alcohol burned gives 2.1 and 2.6 per cent respectively for the oxygen which is of the same order as those for the respiratory quotient.

These experiments show that the Haldane chamber respiration apparatus will give reliable respiratory quotients with adult poultry when the periods are at least two hours in length and a balance sensitive to 0.01 gram with a load of 5 kilograms is used. For shorter periods it is necessary to have a more sensitive balance, although there are a number of periods (of one hour duration) in this series which are satisfactory so far as respiratory quotient is concerned. The greatest difficulty is in weighing the chamber, particularly with a live animal in it. Care should be taken to maintain a uniform rate of ventilation so as to have the composition of the residual air as nearly the same at the end of each period as possible, and to perform the weighing in the same length of time at each period, as unequal duration of weighings results in unequal changes in temperature of the chamber, which affect the weight of the chamber. When these precautions are observed and a suitable balance is used, the method should give trustworthy results in the measurement of respiratory exchange of small animals.

All measurements are made with one instrument (the balance), and therefore, the factors measured should all have the same degree of accuracy. The equipment required can be assembled easily and with the exception of

the chamber can be readily purchased. The technic is not complicated, and therefore, can be acquired quickly. The method has also the great advantage that several sets of apparatus can be used and only one balance is required. In view of the simplicity of the method, both in theory and in equipment required, it is surprising that it has had so little application until recent years.

SUMMARY

Control tests with ethyl alcohol were made with a Haldane chamber respiration apparatus suitable for adult poultry. The average percentage recovery for water, carbon dioxide, and oxygen in 8 experiments of one hour to five hours in duration was 101.8, 98.4, and 99.6 respectively. The average respiratory quotient was 0.659. The mean deviation from average in 29 periods was for water, 4.0; carbon dioxide, 4.5; and oxygen, 5.3 per cent. The mean deviation of the respiratory quotient from 0.659 was 0.016.

REFERENCES

Bertschi, H.: Biochem. Ztschr., 1920, cvi, 37.
Boothby, W., and Sandiford, I.: Physiol. Rev., 1924, iv, 75.
Cori, C. F., and Cori, G. T.: Jour. Biol. Chem., 1926, lxx, 560. Jour. Biol. Chem., 1928, lxxvi, 759.
Cramer, W., and McCall, R.: Quart. Jour. Exper. Physiol., 1917, xi, 61.
Danoff, N.: Biochem. Ztschr., 1919, xciii, 44.
Dittler, R.: Ztschr. f. Biol., 1922, lxxvi, 145.
Fridericia, L. S.: Biochem. Ztschr., 1913, liv, 92.
Haldane, J. S.: Jour. Physiol., 1892, xiii, 419.
Harris, D. T.: Roy. Soc. Proc., 1925, Ser. B. xcviii, 171.
Hédon, L.: Thèse, Montpellier, 1921; Arch. internat. de physiol., 1922, xxi, 45; Arch. internat. de physiol., 1926, xxvi, 329.
Isenschmid: Arch. f. exper. Path. u. Pharmakol., 1920, lxxxv, 271.
Kennaway, E. L., and Pembrey, M. S.: Jour. Physiol., 1912, xlv, 82.
Krogh, A.: The Respiratory Exchange of Animals and Man, 33, Longmans, Green & Co., New York, 1916.
Lee, M. O.: Am. Jour. Physiol., 1927, lxxxi, 492.
Marine, D.: Jour. Metab. Res., 1922, ii, 29.
Marine, D., and Lenhart, C. H.: Am. Jour. Physiol., 1920, liv, 248.
Mitchell, H. H., and Carman, G. G.: Am. Jour. Physiol., 1926, lxxvi, 387.
Mitchell, H. H., and Haines, W. T.: *a*, Jour. Agric. Res., 1927, xxxiv, 549; *b*, Jour. Agric. Res., 1927, xxxiv, 927.
Mutch, N., and Pembrey, M. S.: Jour. Physiol., 1911, xliii, 109.
Nakayamo, K.: Biochem. Ztschr., 1924, cxlviii, 98.
Okumura, T.: Biochem. Ztschr., 1926, clxxvi, 325.
Pembrey, M. S.: Jour. Physiol., 1894, xv, 401; Jour. Physiol., 1895, xviii, 363; Jour. Physiol., 1901, xxvii, 66; Jour. Physiol., 1903, xxix, 195.
Pembrey, M. S., Gordon, M. H., and Warren, R.: Jour. Physiol., 1894, xvii, 331.
Pembrey, M. S., and Gürber, A.: Jour. Physiol., 1894, xv, 449.
Pembrey, M. S., and Spriggs, E. I.: Jour. Physiol., 1904, xxxi, 320.
Reiss, M., and Weiss, R.: Klin. Wchnschr., 1925, iv, 2207.
Také, N. M.: Jour. Infect. Dis., 1923, xxxii, 138.
Terroine, E. F., Trautman, S., and Schneider, J.: Ann. de Physiol., 1926, ii, 468.
Terroine, E. F., and Trautman, S.: Ann. de Physiol., 1927, iii, 422.

A LAMP FOR THE COLORIMETER OR MICROSCOPE*

By H. A. Davenport, M.D., Chicago

ARTIFICIAL light suitable for either colorimetric or microscopic work should be a good imitation of daylight, and be supplied in a diffuse beam which is large enough to cover the reflector or mirror of the instrument used. Such a source of light is produced by the lamp described here.

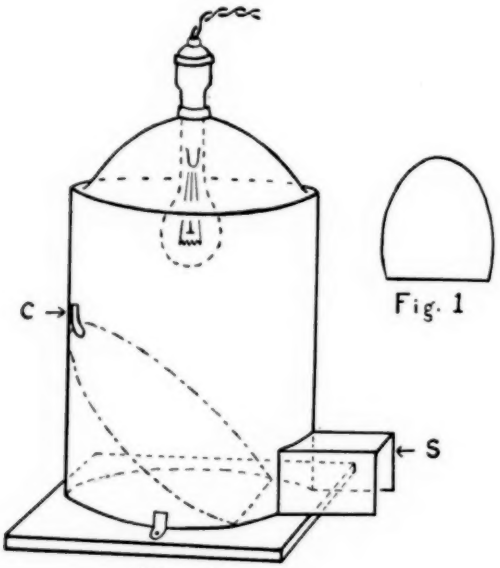

Fig. 1

Fig. 2

The body of the lamp is made from a standard two gallon gasoline or kerosene can. The spout is removed and the hole covered by soldering on a metal patch. A standard brass-shell electric light socket is soldered into the collar which is normally covered by the filler cap of the can. The bottom of the can is cut out, and a rectangular hole about 2½ inches high by 4 inches wide cut in the side at the lower edge. A metal shield (*s*, Fig. 2) shaped like a broad inverted U is soldered around the edge of the rectangular hole. This shield should project 3 or 4 inches from the side of the can. A reflector, semi-oval in shape, is made from any thin material which has a plane, smooth surface. If metal is used, care should be taken that it is flat. The reflector should be fitted at an angle of 45° and a small metal clip (*c*, Fig. 2) soldered to the inside wall of the can opposite the opening and at the height necessary to hold the top of the reflector. Bottom supports for the reflector are not nec-

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essary if it fits well. Two small brass angle irons with screw holes in their ends are soldered to the sides of the bottom for attaching it to the base.

The inside of the can and the reflector are painted (two coats) with aluminum paint. The outside will probably tire the eyes least if painted dull black.

A base is made of a piece of board 10 inches square. It should be just thick enough to bring the opening of the lamp level with the reflector of the microscope or colorimeter, or varying heights may be obtained by "shimming up" the lamp with additional squares of thick cardboard.

The order of assembly is as follows: Insert a 100 watt blue glass (daylight) bulb into the socket. Adjust the reflector so that the beam is thrown straight out the "tunnel" made by the shield, and screw the can to the base.

Fig. 1 illustrates the shape of the reflector. Fig. 2 is a skeleton diagram of the completed apparatus showing the placement of the reflector.

The device can be constructed by a tinner in an hour or two at a cost of a few dollars, and will prove to be a handy substitute for daylight in the laboratory. The color value given for blue will be found much superior to those given by an ordinary electric light. Used for microscopic work it will be found superior to the substage lamp.

Points to be emphasized are: use aluminum paint (not white), use a blue glass, nitrogen filled bulb, and set the reflector at such an angle that both sides of a colorimeter field are illuminated equally.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

TISSUE STAIN: A Modified Methylene Azure B Stain for Sections of Human Hematopoietic Organs, Richter, M. N. Arch. Path. and Lab. Med., 1927, iv, v, 773.

1. Fix (A) pieces not more than 2 mm. thick by Ellermann's method:

Twenty-four hours in freshly prepared mixture of Zenker (without acetic acid)	9 parts
Neutral formalin (40 per cent formaldehyde)	1 part

2. Wash in running water, from twelve to twenty-four hours.
3. Embed in celloidin (B) (or paraffin).
4. Attach sections to slide by albumin and remove celloidin with absolute alcohol and ether (b) (or paraffin with xylene).
5. Absolute alcohol, one minute.
6. Ninety-five per cent alcohol containing tincture of iodine, fifteen minutes.
7. Eighty per cent alcohol until iodine is removed.
8. Water: several changes to remove alcohol.
9. Stain fifteen minutes, while steaming (C) in freshly prepared mixture of

Erythrosin, 1 per cent aqueous	5.0 c.c.
Neutral formalin	0.25 c.c.

Eosin or phloxine may be substituted for erythrosin.

10. Rinse in distilled water.
11. Stain from one to five minutes in:

Methylene azure B bromide	0.1
Methylene blue U.S.P. medicinal	0.1
Distilled water, or preferably buffer solution, P_H 7.5	100.0

The buffer is made by adding 15 c.c. of a fifteenth molecular solution of primary potassium phosphate (9.078 gm. to 1 liter of distilled water) to 85 c.c., of secondary sodium phosphate solution (11.876 gm. to 1 liter).

12. Flood from dropping bottle with several changes of absolute ethyl alcohol, until stain ceases to come out (ten seconds at least). Avoid water and lower alcohols.
13. Differentiate in:

Clove oil	3 parts
Absolute ethyl alcohol	1 part
Colophonium, sat. alc. solution	a few drops

Control the differentiation under the microscope.

14. Place for thirty seconds in (D):

Xylene	9 parts
Amyl alcohol	1 part

The differentiating fluid must be thoroughly removed.

15. Pure amyl alcohol, until ready to mount.
16. Mount in euparol, preferably the green variety.

Results

The nuclei are a deep blue-black and the chromatin is sharply stained.

The nucleoli are distinct. The cytoplasm is various shades of lavender, red or blue, depending on cell types. The eosinophil granules are red, the eutrophil granules slightly

darker red, and the basophil granules blue. The centrosomes are a bright red. The spindle fibers are usually red, and sometimes are purple. The fibrin is red or purple, depending on the degree of decolorization. The bacteria are blue.

LIVER FUNCTION: Rose Bengal Test for Liver Function, Epstein, N. N., Delprat, G. D., and Kerr, W. J. Jour. Am. Med. Assn., 1927, lxxxviii, 1619.

The authors report the results of their continued study of the test described by them in 1923, the present report summarizing the findings in more than 500 cases.

The modified technic follows:

Injection: A vein in the cubital fossa is selected, and 10 c.c. of a 1 per cent solution of rose bengal in physiologic sodium chloride solution is injected, and the needle washed through slowly with from 5 to 10 c.c. of salt solution, which is held ready in a fresh syringe. The needle is left in the vein, and at exactly two minutes after the injection of the dye a sample of blood (10 c.c.) is withdrawn from the needle into a clean syringe and discharged into a centrifuge tube containing a few crystals of potassium oxalate. The tube is carefully inverted two or three times. The needle is again washed by slowly injecting from 5 to 10 c.c. of the salt solution, which maneuver prevents the clotting of blood in the needle. At eight and sixteen minutes, respectively, from the time of injection, samples of blood are withdrawn and collected in an identical manner. The needle is then withdrawn from the vein in the arm. It is well to advise the patient that the appearance of the dye in the stools will impart to them a distinct red color, since the unexpected appearance of the red stools may cause considerable alarm.

As soon as possible after collection, the blood samples are centrifuged at a speed of 2,000 revolutions per minute for thirty minutes.

Ten c.c. record syringes and number 16 gage record needles are convenient. Rough handling of the blood samples is apt to cause some hemolysis of the red cells and to interfere with the colorimetric readings.

Colorimetric Analysis: Five cubic centimeters of the plasma of each of the samples is separately mixed with 10 c.c. of physiologic sodium chloride solution. The diluted plasma of the two minute sample is placed in the wedge of the Hellige colorimeter. This is used as the standard against which the eight and sixteen minute diluted samples are read.

Since the two minute sample represents the highest concentration of the dye in any individual, following the injection of a fixed amount of the dye into the circulation, it may be regarded as 100 per cent retention, and the colorimetric readings obtained by comparing the eight minute and sixteen minute specimens with it represent direct percentages of dye retained. Before making colorimetric readings, it is necessary to standardize the Hellige colorimeter used and to correct for any inaccuracies present. The colorimeter is standardized by comparing one sample of plasma against itself; the reading should be 100.

The colorimetric readings thus obtained indicate the ratio between the first or control sample and the subsequent samples. This obviously indicates the speed with which the dye leaves the circulation.

While the two minute sample is regarded as representing the highest concentration of the dye in an individual at any one time, it is probable that some of the dye is removed from the circulation before the collection of the two minute sample; but this is not of great practical importance if care is taken to withdraw the blood at exactly two minutes. Furthermore, we are not as much concerned with the actual quantity of dye in the circulation as with the ratio of the percentage of dye in circulation at two distinct intervals of time, which in reality is its rate of disappearance. For this reason, it is not essential that the solution of dye injected be exactly a 1 per cent solution nor that the dose be exactly 10 c.c. Although considerable latitude may be taken in regard to the amount of dye injected, it would be advisable to inject approximately 100 mg. in the average sized adult. The dose may be varied according to the size of the patient, for some endeavor should be made to keep the initial concentration of the dye approx-

imately the same. The dosage has varied from 3 c.c. of a 1 per cent solution in children to 15 c.c. in large adults. In normal individuals, the eight minute sample is usually 50%, varying from 40 to 60 per cent, and the sixteen minute sample varying from 23 to 30 per cent. These percentages represent the amount of dye retained in the circulation.

The results may be thus summarized:

Normal persons absorb rose bengal through the liver from the blood stream very rapidly so that at the end of sixteen minutes only a trace remains in the blood plasma; that is, rarely more than 25 per cent of the amount injected.

In chronic cholecystitis the elimination is usually within normal limits, and the test is not an aid in the diagnosis of the condition.

In cases of obstructive jaundice, catarrhal jaundice and arsphenamine icterus, the delay in the elimination of the dye is always very definite, being greatest in obstructive jaundice. The test is a valuable aid in detecting these conditions and in following their clinical course. Before the jaundice begins to clear clinically the improvement in liver activity can be demonstrated by its increased ability to eliminate rose bengal. Following operations for the relief of obstruction of the common duct, the liver function improves markedly. The test may be used as an aid in determining the patency of a cholecystoduodenostomy, as the dye can be readily identified in the duodenal contents. It has proved very valuable in following the course of an arsphenamine hepatitis.

In cirrhosis of the liver, the impairment of liver function is in direct relation to the amount of scarring in the liver and to the encroachment on the functional reserve of the liver. An advanced case of cirrhosis always shows a marked delay in the excretion of rose bengal.

The test is of particular value in the differential diagnosis when ascites is the outstanding symptom. In cardiac failure, in tuberculous peritonitis and in carcinomatosis of the peritoneum, little change from the normal liver function has been noted, while on the other hand, when cirrhosis of the liver is present, there is a very marked impairment of liver function.

In metastatic malignancy of the liver, the conditions are variable and seem to depend on the amount of liver tissue which can function. Discrete scattered metastatic nodules in the liver do not interfere with its ability to eliminate the dye, but diffuse replacement of the liver tissue by carcinoma very definitely causes a retention of the dye in the blood stream.

Chronic passive congestion of the liver does not seem to prevent the absorption of rose bengal, and the curves are within normal limits.

Observations in patients in the various stages of syphilis have proved interesting, especially in those with a very florid type of early generalized infection. In a small percentage of these a definite delay in the excretion of the dye has been noted, and probably indicates the presence of an acute syphilitic hepatitis in spite of the clinical silence of the liver. Long-continued treatment with arsphenamine and mercury does not disturb the liver activity. Arsphenamine dermatitis unaccompanied by icterus shows elimination of rose bengal within normal limits.

Acute infections of the liver cause a marked impairment of liver function.

Nephritic toxemias of pregnancy do not cause delay in the elimination of rose bengal from the blood stream.

Continued application of the test confirms previous observations as to its value as an aid in diagnosis and in estimating the prognosis of certain diseases of the liver.

NERVE TISSUE STAIN: Stain Technic for Nissl's Granules Following Alcohol-Formol Fixation, Bean, R. J. Stain Technology, 1927, ii, 56.

1. Nerve tissue is fixed 2-4 hours in a 5 per cent solution of strong formalin in commercial alcohol.

2. If dehydration is perfect, either chloroform or xylol may be used as a clearing agent.

3. A slow method of paraffin infiltration is advisable.

4. Sections should be cut 10-12 microns in thickness.

5. Coplin staining jars should be annealed by placing them on a rack in a pan of cold water, bringing the water to the boiling point, and allowing the jars to stand in boiling water for twenty minutes.

6. One per cent aqueous solutions of either methylene blue or Grubler's Neutral Roth are used as specific stains for Nissl's granules.

7. These stains are heated to boiling in a beaker, the slides are placed in the Coplin jars which are partially submerged in boiling water, and the hot stain poured into the jars. The flame beneath the water-bath is turned down and the slides left for 20 minutes.

8. The excess of primary stain is washed off in 25 per cent and 50 per cent alcohol and the slides passed rapidly through the alcohol series to absolute alcohol, and finally to xylol.

9. When counterstaining is desired, nigrosin in 1 per cent aqueous solution, methyl orange, saturated solution in 50 per cent alcohol, or a 0.5 per cent solution of eosin in 50 per cent alcohol are recommended. These stains are used cold, and the slides are merely dipped in them after the excess of primary stain has been washed out in 25 per cent and 50 per cent alcohol.

10. If a cold primary stain is desired, a saturated solution of thionin in distilled water, acidified with 1 per cent carbolic acid, will prove specific for the Nissl substance. Sections should be stained 5-10 minutes in thionin, then passed rapidly through to absolute alcohol, and xylol. The same counterstains may be used as in the hot method.

11. Sections prepared by the hot method show little tendency to fade after ten years' use.

12. Excepting neutral red, all the stains used in this technic are carried by the National Aniline and Chemical Company and are satisfactory. Coleman and Bell natural red may be substituted for Grubler's Neutral Roth with good results.

SPIROCHETA PALLIDA: A Modified Stain for, Cerqueirs, A. Brazil Med., 1927, xli, 258.

Stain:

Tannin C. P.	5 gm.
Glacial acetic acid	4 c.c.
90 per cent alcohol	50 c.c.
Distilled water	50 c.c.

Chancre serum is smeared and fixed in 90 per cent alcohol 1 minute and without drying covered with the stain. The slide is heated until a little vapor is given off, washed in running water, and covered with Ziehl-Neelson carbolfuchsin and steamed for 10-20 seconds. Wash.

Treponema are pinkish violet.

BLOOD GROUPING: A New Method of Direct Matching, Clemens, J. Zentr. f. Chirurg., 1926, liii, 3032.

The following rapid method is proposed for compatibility tests:

One or more drops of the recipient's blood are placed upon a slide and an equal volume of chloroform added, the mixture being spread to twice the size of the original drop. The chloroform hemolyzes the blood and produces a clear, red serum. If necessary more chloroform may be added and poured off. The procedure thickens the blood somewhat.

A drop of the donor's cells is then added to the hemolyzed serum and the mixture observed for agglutination.

The procedure is repeated testing the recipient's cells against the donor's hemolyzed serum.

PANCREATIC ENZYMES: Estimation of, Martin, L. Arch. Int. Med., 1927, xxxix, No. 3, p. 343.

Determinations are made preferably upon B bile or, if this is turbid, upon clear A bile.

After thorough centrifugalization, 1 c.c. is placed in 19 c.c. of buffer solution P_H 7.7 and 1 c.c. of the 1:20 mixture in 29 c.c. of the same buffer. This makes a 1:600 dilution. These two dilutions are sufficient.

The buffer solutions are prepared as follows:

An M/15 solution of primary potassium phosphate which contains 9.080 gm. KH_2PO_4 in 1 liter of solution.

An M/15 solution of secondary sodium phosphate which contains 11.876 gm. Na_2HPO_4. $2H_2O$ in 1 liter of solution.

Nine cubic centimeters of the secondary are added to 1 c.c. of the primary to make P_H 7.731.

Into three Folin and Wu blood sugar tubes are placed respectively, 1 c.c. of the 1:20 dilution measured from an accurate 1 c.c. pipette; 0.1 c.c. from a 0.1 c.c. pipette, of the 1:20 dilution with 0.9 c.c., standard buffer, 1 c.c. of the 1:600 dilution. To each tube is added 1 c.c. of 1 per cent starch solution made up by boiling as directed. This is incubated for thirty minutes in a water-bath, 38° to 40° C., and 2 c.c. alkaline copper sulphate of Folin and Wu added and carried out as directed by them for blood sugar determination. In normal cases the digestion in tubes 1 and 2 will be practically complete, certainly in 1. Tube 3 will be the only one necessary to read. If this reduction is low, it will be safe to take the 1:200 tube as representing the true pancreatic activity. This has been found to be the case as shown in the foregoing determinations in a series of five tubes with progressively smaller amount of each biliary fraction in 1:600 dilution have been conducted. The milligrams of dextrose produced from one c.c. of starch are then calculated.

To arrive at a unit of comparison this simply necessitated the multiplication of the amount found in tube 3 by 100, in tube 2 by 33.3 and in tube 1 with a dilution of 1:20 by 3.3. For instance, in tube 3 the colorimetric reading of $\frac{\text{Standard}}{\text{Unknown}} \times$ amount of glucose in standard $\times$ 100 = mg. of glucose, or to give an example using standard 2 of glucose working standard; $20/15 \times 0.4$ mg. $\times$ 100 = mg. This represents 100 times the amount of glucose produced from one c.c.—1 per cent starch—by the action of 1:600 dilution of pancreatic enzyme as obtained by duodenal drainage.

MILK: Can B. Coli be Used as an Index of the Proper Pasteurization of Milk? Swenarton, J. C. Jour. Bact., 1927, xiii, No. 6, p. 416.

Sixteen pasteurizing plants were studied with respect to the B. coli contents of the pasteurized milk.

The B. coli content of the pasteurized milk from the different plants was found to vary considerably.

The control charts from the plants whose milk was high in B. coli show improper heating or irregularity of procedure. There is a definite correlation between B. coli content and procedure as indicated by the control charts.

A test for B. coli in pasteurized milk can be used to good advantage in checking up on plant performance.

A standard is proposed for the maximum B. coli content of a properly pasteurized milk as follows:

The standard portion of milk shall be 0.1 c.c. The standard sample shall consist of five standard portions of 0.1 c.c. each.

Of all the standard 0.1 c.c. portions examined, not more than 20 per cent shall show the presence of organisms of the B. coli group.

Occasionally three or more of the five equal 0.1 c.c. portions constituting a single standard sample may show the presence of B. coli. This shall not be allowable if it occurs in more than (a) 10 per cent of the standard samples when ten or more samples have been examined; or in (b) one standard sample when less than ten samples have been examined.

ALCOHOL IN TISSUES: The Quantitative Determination of Alcohol in Human Tissues, Gettler, A. O. and Tiber, A. Arch. Path. and Lab. Med., 1927, iii, 75.

The tissue, as soon as it is removed from the body, should be placed in a tightly closed jar and placed in a refrigerator. When ice cold, 500 gm. are weighed out, quickly ground up and placed in a 2 liter flask. To this are added 600 c.c. of water, 5 c.c. of a saturated solution of tartaric acid and 1 c.c. of white mineral oil. This mixture is now distilled with steam. A long, well cooled condensor should be used, and the distillation should be continued until exactly 800 c.c. has been collected. The distillate is well mixed and used in the following procedure:

Twenty grams of potassium bichromate and 40 c.c. of concentrated sulphuric acid are placed in a 500 c.c. distilling flask. Three hundred cubic centimeters of the distillate obtained above are now added and the contents mixed well. The flask is then connected to a long, well cooled condensor by means of a Hopkins distilling head, and the distillation started. The heat must be so regulated that it will take from forty-five to fifty minutes to collect exactly 250 c.c. of distillate. After thoroughly mixing, 50 c.c. of this distillate is titrated with twentieth normal sodium hydroxide solution, using phenolphthalein as an indicator. From this titration figure, the amount of alcohol present in 1 kilogram of brain tissue can be easily calculated as follows: (c.c. twentieth normal alkali $-$ 1.32) x 71.58 = mg. C_2H_5OH per kilogram of tissue. The figure ($-$ 1.32) holds only for brain tissue. For other tissues, one must determine experimentally the corresponding blank.

INTOXICATION: The Alcoholic Content of the Human Brain, Gettler, A. O. and Tiber, A. Arch. Path. and Lab. Med., 1927, iii, 218.

For the qualitative and quantitative determination of alcohol, the brain is of first importance.

The normal alcoholic content of human brain material is less than 0.0025 per cent.

The alcoholic content of the brain in persons who have partaken of alcoholic beverage ranges between 0.005 and 0.6 per cent.

All patients having an alcoholic content below 0.1 per cent (designated as "one plus") show no abnormal physiologic effects.

Patients with an alcoholic content above 0.1 per cent and up to 0.25 per cent (designated as "two plus") show some physiologic disturbance, as evidenced by increased aggressiveness, and more or less loss of sense of care. None of these patients, however, shows unbalanced equilibrium, which is commonly called intoxication.

When the alcoholic content rises above 0.25 per cent and up to 0.4 per cent (designated as "three plus") and still higher up to 0.6 per cent ("four plus"), the equilibrium of the person becomes unbalanced—a condition generally know as intoxication.

The degree to which any person is affected does not depend on the quantity of alcohol consumed, but on the amount of alcohol present in the brain at the time.

AMEBA: A Simplified Method for the Cultivation of Endameba Histolytica, Craig, C. F. Am. Jour. Trop. Med., Sept. 1926, vi, No. 5, p. 333.

A medium consisting of Locke solution containing one part to seven of inactivated human, horse, or rabbit blood serum has been found very efficient in the cultivation of Endameba histolytica. The best results were obtained when inactivated human blood serum was used.

The excellent growth of Endameba histolytica obtained in this medium demonstrates that coagulated egg or blood agar slants used as a substratum, are not essential for the growth and development of this species, and that neither egg nor blood are essential as a part of the food supply of this species.

In this medium Endamoeba histolytica has remained alive and motile for a period of eleven days and successful transfers have been obtained in the medium after a period of eight days. The best results in transferring cultures are obtained by making transfers every twenty-four or forty-eight hours.

Transfers of Endameba histolytica, in this medium, made at intervals of twenty-four and forty-eight hours, have been continued for over three months and the ameba have not changed in morphology or in their capability for reproduction during this time.

In a second paper (Observations Upon the Cultivation of Endamoeba Histolytica, Craig, C. F. Amer. Jour. Trop. Med., November 1926, 461) Craig gives the following still further simplified formula:

The salt solution-serum medium is prepared by adding to seven parts of normal salt solution (0.85) which has been autoclaved, one part of human blood serum, inactivated by heating at 56° C. for one half hour. The mixture is then filtered through a Mandler filter, tubed, and kept in the incubator at 37° C. to determine sterility. If sterile, it should be stored in the incubator at 37° C. until used. To inoculate, a small lump of fecal material is mixed with the medium and examined at the end of twenty-four hours, the inoculated tube being kept in the incubator at 37° C. Daily transfers should be made if the culture shows the organism.

QUININE IN THE BLOOD: On the Estimation of Minute Quantities of Quinine In the Blood, Roy, A. C. Indian Jour. Med., 1926, xiv, 129.

Roy has used with satisfaction the following method described by Acton and King:

Five c.c. of blood were laked with 5 c.c. of distilled water in a beaker. Then 20 c.c. of saturated ammonium sulphate containing 0.6 per cent H_2SO_4 were added to it and also 5 grams of pure solid ammonium sulphate and the contents mixed by stirring. The beaker was then heated in a water-bath with constant stirring of its contents, when a partial coagulation of the proteins took place. The mixture was then brought to the boil in a free flame and filtered through a Gooch's crucible, packed with a layer of asbestos, covered with a perforated porcelain filter disc, and over the latter a filter paper, in order to prevent the coagulum passing into the asbestos. The coagulum on the crucible was thoroughly pressed down and when the filtration was complete, the coagulum was transferred into the beaker again, and treated with another 20 c.c. of acidified ammonium sulphate solution. It is preferable to macerate the coagulum first with 3 c.c. of the acidified ammonium sulphate solution to a fine paste, and then in each case to add the remaining 17 c.c. washing the sides of the beaker with it. The contents of the beaker were well mixed, again brought to the boil and filtered; this process being repeated 10 times in all.

The entire filtrate about 200 c.c. was transformed to a 250 c.c. well stoppered cylinder, and 10 c.c. of pure ammonia solution added, followed by 20 c.c. of specially purified ether. The contents of the cylinder were thoroughly shaken and then allowed to settle. The top ethereal layer was transferred to a test tube of pyrex glass 6 in. x 1 in. placed over hot water, by means of a 1 c.c. pipette provided with a rubber teat and evaporated away. On shaking with further successive quantities of 15, 15, 10 c.c. of ether, the whole of the quinine was practically extracted, the ether being in each case transferred into the boiling tube and evaporated away. To the quinine that was left in the boiling tube, 10 c.c. of saturated ammonium sulphate solution were added, and the quinine was brought into solution by bringing it to the boil in a heated brine bath. The solution was then rapidly cooled in running water, and 5 c.c. of it were taken in one of a series of standard 13 mm. tubes. The other tubes contained 5 c.c. each of various standard solutions of quinine made by adding the requisite quantities of a 1 in 100,000 quinine

in saturated ammonium sulphate solution, so as to obtain solutions differing in strength by .005 mg. of quinine, the difference being made up with saturated ammonium sulphate.

At this point Roy introduces a modification in the use of N/100 iodine solution as an indicator.

0.5 c.c. of this solution is added to each tube and the contents mixed.

It was then ascertained between which of the known solutions differing by .005 mg. of alkaloid content the unknown lay, and then the remaining 5 c.c. of the quinine solution was matched against another set of test tubes containing known quantities of quinine (1 in 100,000) differing in strength by .001 mg. between the range found in the preliminary trial, for more accurate determination.

He found that:

1. Tanret's reagent does not give good results in concentrations higher than 0.025 mg. in 5 c.c. specially when the matching tubes are heated in the brine bath and then cooled, as recommended by Ramsden and Lipkin.

2. On using Wagner's reagent the solutions become colored too deep a yellow for making delicate comparisons.

3. An acidified N/100 iodine solution has been found to give very good results in detecting a difference of 0.002 mg. in the alkaloid contents between the ranges of 0.045 mg. in 5 c.c. to 0.03 mg. in 5 c.c. and a difference of 0.001 mg. between 0.03 mg. in 5 c.c. to 0.001 mg. in 5 c.c. concentrations.

4. With 5 c.c. as total volume, even the presence of 0.0005 mg., i.e., 1 in 10,000,000, can be detected when matched against 5 c.c. of a saturated ammonium sulphate solution alone, to which the same quantity of the reagent has been added.

5. With 2 c.c. as total volume, even the presence of 0.0001 mg., i.e, 1 in 20,000,000, can be detected provided it is matched against a blank.

SCARLET FEVER: Amato Bodies in Scarlet Fever, Toomey, J. A. and Gammel, J. A., Am. Jour. Dis. Child., 1927, xxxiv, No. 5, 841.

The investigations of the authors lead them to conclude that Amato bodies are not always present in scarlet fever and may be found in other diseases, although they are present more commonly in scarlet fever than in any other infection.

PNEUMOCOCCUS: A Specific Flocculation Reaction Occurring Between Alcoholic Extracts of Pneumococci and Anti-Pneumococcus Serum, Jungeblue, C. W., Jour. Exper. Med., 1927, xlv, No. 2, p. 227.

Antigens: The bacterial antigens were prepared by extracting the centrifugalized, washed sediment of 1500 c.c. of an 18 hour pneumococcus broth culture in 40 c.c. of absolute or 95 per cent ethyl alcohol for a period of 4 weeks, at room temperature. The extracts were then filtered through paper and were ready for use. Antigens obtained by extracting the bacterial sediment for 2 hours with boiling alcohol in a reflux container were found to be only slightly weaker in flocculating power than those prepared by the first method.

To one part of benzoin tincture are added twelve parts of alcoholic bacterial antigen. This initial mixture is suspended in 0.85 per cent salt solution by adding quickly 25 c.c. of the salt solution to 0.4 c.c. of the initial mixture. It is important to add the diluent very quickly. Experiments in which buffered salt solutions of different P_H values were used in preparing this suspension, indicated a neutral medium as the most desirable for the reaction. The colloidal supension thus obtained is slightly opaque and just on the verge of spontaneous flocculation; if left standing at room temperature or shaken vigorously it will flocculate by itself. In order to insure conditions of maximum sensitivity, it is necessary to determine for each new antigen and new benzoin tincture the optimum balance between bacterial antigens, benzoin, and salt solution. A constant volume of 2.5 c.c. of this suspension is mixed with varying amounts of serum, starting with

0.5 c.c. and ending with 0.05 c.c. It is not advisable to test smaller amounts than 0.05 c.c. because it was found that less than 0.05 c.c. of normal serum is insufficient to stabilize the suspension; the comparable range of specific flocculation is thus limited to the amounts indicated. The tubes are incubated, half immersed in a water-bath at 40° C. since this temperature was found to provide conditions of the greatest sensitivity, maintaining at the same time the highest degree of specificity; it was observed that normal horse serum flocculated slightly above 46° C. Readings are made after ½ hour, 1 hour, and 2 hour intervals to determine the degree of flocculation. Occasionally, another reading is made on the following day.

The reaction appears to be species-specific. It is not strictly type-specific, as slight or moderate cross-reactions occurred between Type I serums and Type II and Type III extracts.

The flocculating power of the serum from five horses undergoing immunization with pneumococcus, Type I, did not develop to any extent before the end of the 4th or 5th month.

In the case of two of these horses in which it was possible to carry out parallel tests on a larger number of subsequent bleedings until the end of immunization, some relationship was suggested between the flocculating power and the protective titer as ascertained by the routine method of standardization in mice.

BACTERIOLOGICAL TECHNIC: A Method for the Detection of Changes in Gelatin Due to Bacteria, Frazier, W. C. Jour. Infect. Dis., 1926, xxxix, No. 4, 302.

The method is as follows:

The gelatin-agar medium used for the test is made as follows: In 100 c.c. of distilled water are dissolved NaCl 5.0 gm., KH_2PO_4 0.5 gm., K_2HPO_4 1.5 gm.

Four grams of bacto-gelatin are dissolved in 400 c.c. of distilled water, and to this solution are added dextrose 0.05 gm., bacto-peptone 0.1 gm., beef infusion 5.0 c.c.

The two solutions are poured together, heated in a steamer, and then mixed with 500 c.c. of 3% washed agar. The P_H is adjusted to about 7.0. The medium is placed in tubes or flasks and sterilized in the autoclave.

Plates of the gelatin-agar medium are poured and allowed to harden. Duplicate plates are inoculated on the surface of the agar at the center so as to form a giant colony of the organism, and are incubated for two or three days at the optimum temperature of the organism. In general, incubation for 48 hours at 30° C. was found to be satisfactory for the organisms studied.

After incubation, one plate is flooded with a 1% solution of tannic acid, while the duplicate plate is flooded with an acid solution of bichloride of mercury of the following composition: $HgCl_2$ 15 gm., HCl (conc.) 20 c.c., and water 100 c.c.

If the gelatin has been changed, a clear zone will appear about the giant colony on the plate flooded with acid bichloride of mercury solution, surrounded by the cloudy precipitate of unchanged gelatin. The reaction is slow, and 15 to 30 minutes should be allowed for its completion.

The plate flooded with tannic-acid solution will present an appearance that will vary with the amount and degree of action of the bacteria on the gelatin. An organism that acts on gelatin with little or no increase in amino-nitrogen gives a white precipitate about the colony, heavier than the precipitate of gelatin throughout the rest of the plate. With some groups of organisms this precipitate is very heavy and white, with a distinct edge. If there has been considerable decomposition of the gelatin, there is a clear zone about the colony, surrounded by a distinct white ring. This reaction takes place rapidly.

A modification of Sorensen's formol titration method was devised for testing for an increase in amino nitrogen, for it was found that after the organisms had been grouped by the gelatin-agar plate method they could be further subdivided on the basis of whether or not they decomposed gelatin with an increase in amino-nitrogen. The

"amino-P_H" method, as it has been termed, has the advantages of requiring no standard acid or alkali and of using only 1 c.c. of the solution to be tested.

To 1 c.c. of the solution to be tested are added 5 c.c. of distilled water and a drop of phenolphthalein indicator. The tube containing 1 c.c. of the control is brought to a faint pink color; that is, to a P_H of about 8.4 by the addition of dilute alkali. The tubes containing solutions to be tested are then matched to the pink color of the control; that is, brought to the same P_H. Then to each tube is added 0.5 c.c. of formaldehyde solution (about 37%) which has previously been made pink to phenolphthalein. If the pink color disappears from the control solution, dilute alkali is added drop by drop until the pink color reappears. The same number of drops of the alkali are then added to each of the other tubes. If the tubes are pink no increase in amino-nitrogen has taken place. The P_H of the liquid in the colorless tubes is determined colorimetrically by the addition of phenol red or brom-thymol blue indicator, and this value is called the "amino-P_H number."

This method has been very useful in detecting small increases in amino-nitrogen and also gives a rough idea of the amount of that increase. It is useful in testing clear, synthetic medium like the gelatin solution but cannot be used, of course, in colored solutions.

The paper is illustrated with 12 microphotographs illustrating the reactions.

LEPTOSPIRAS: A Method for the Isolation of Leptospiras from Water. Bauer, J. H., Am. Jour. Trop. Med., 1927, vii, No. 3, p. 177.

The water was filtered first through paper, then through Berkefeld V and finally through Berkefeld N filters. The filtrate served as the inoculum as well as a constituent of the medium used. This medium was prepared on the same principle as Noguchi's semi-solid leptospira medium with the modification that approximately 1 per cent of fresh defibrinated guinea pig's blood was used in place of 10 per cent of rabbit's serum. The various ingredients were mixed in the following proportions:

Filtered water	200 c.c.
2 per cent nutrient agar, melted and cooled to 45° C.	20 c.c.
Fresh defibrinated guinea pig's blood	2 to 3 c.c.

This mixture was placed in a number of ordinary test tubes, approximately 7 c.c. in each, and kept at room temperature in the dark. The temperature in the laboratory varied between 22° and 35° C.

If leptospiras were present in the water, growth usually occurred between the sixth and tenth day of incubation. It was clearly visible to the naked eye as a grayish haze and a partial decoloration of the hemoglobin, extending about 1 cm. below the surface of the medium. When examined under the dark-field microscope, this haze showed large numbers of leptospiras. In the examination of positive samples of water, it was usual to find one or more tubes containing pure cultures of leptospiras, while most of the tubes showed mixed cultures of leptospiras and other spirochetes, or only several types of these other spirochetes. If the medium was placed in a large number of small test tubes, about 2 or 3 c.c. in each, most of the tubes usually contained only one kind of organism.

SPIROCHETA PALLIDA: Demonstration of in Single Microscopic Sections, Dieterle, R. R., Arch. Neurol. and Psych., 1927, xviii, 73.

A reliable technic has been developed which has been found more satisfactory than any other method on account of its reliability, simplicity, and time-saving features.

1. After fixation and cutting, place the preparations in a 1 per cent solution of uranium nitrate in 70 per cent alcohol at 55° C. for one-half hour.

2. Wash for a moment in distilled water.

3. Pass the sections through 96 per cent alcohol, U. S. P.

4. Handling the sections individually, place then in an absolute alcoholic solution of gum mastic (10 per cent) long enough to allow thorough infiltration—about 30 seconds. Immerse section for an instant in 96 per cent alcohol.

5. Transfer to distilled water.

6. Silver for from 1 to 6 hours at 55° C. in 1 per cent aqueous silver nitrate solution, carrying out this procedure without exposure to light.

7. Wash for a moment in water.

8. Develop in the following reducing solution for from 5 to 15 minutes:

Hydrochinone	1.5 gm.
Sodium sulphite	0.25 gm.
Neutral solution of formaldehyde (Merck's Neutral "Blue Label") 40 per cent solution	10. c.c.
Acetone	10. c.c.
Pyridine	10. c.c.
Water, to make	90. c.c.

Mix and dissolve these and then add 10 c.c. of 10 per cent absolute alcoholic mastic solution to make the mixture milky.

9. Wash for a moment in distilled water.

10. Dissolve out the mastic and dehydrate by transferring the sections to 96 per cent alcohol, U. S. P., and then to acetone. Clear in xylene and mount in Canada balsam.

The distilled water must be free from chlorides and all glassware must likewise be clean. Frozen and celloidin sections are handled with glass hooks and cover glasses with ordinary forceps.

The final result produces sections that are a golden yellow to golden brown, according to thickness. The spirochetes are brown to black in proportion to the heaviness of impregnation.

GONOCOCCUS: A Method for Preserving the Life of Gonococcus Cultures, Krautz, W., Dermat. Wchn., 1927, lxxxv, 1252.

Utilizing the observation of Kaudiba that the life of gonococcus cultures may be prolonged if only a single spot on a plate is touched with the inoculating needle, Krautz makes such a "spot inoculation" on an ascites-agar plate which is then inverted over the lid of a larger dish containing a bichloride solution to prevent contamination and supply moisture.

A single large colony results from which transplants may be made, according to Krautz, for as long as 3 months.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Diseases of the Skin and Syphilis**

THE aim of this book is to present a teaching treatise on dermatology for the use of the student and general practitioner. The object sought was to so present the pathologic, the clinical and therapeutic facts as to render the clinical clear on the basis of the pathologic, and the therapeutic sound on the basis of the pathologic and clinical.

The plan of the volume is thus both ambitious and practical. Tabular presentations of differential diagnosis are frequently used and in the presentation of formulas, the exact purpose of each ingredient is indicated, a new and useful departure.

The volume can be recommended not only to the student but to the practitioner at large.

The book is profusely illustrated, mainly with photographs which are excellently reproduced.

American Health Congress†

A REPORT of the Proceedings of the Forty-first Annual Meeting of the Conference of State and Provincial Health Authorities of North America held in 1926.

Numerous papers on a variety of subjects concerned with public health, of especial interest being a report of the plague situation in California; the need of epidemiologic research in trachoma; and a report concerned with the conservation of vision.

Annals of the "Pickett-Thomson" Research Laboratory‡

THIS publication lists the activities of these laboratories founded by Mr. F. N. Pickett and Dr. D. Thomson and affiliated with St. Paul's Hospital, London.

The publication appears irregularly and is devoted mainly to bacteriology, protozoology, and biochemistry and to recording photographically the whole range of bacteria and protozoa.

In Volume I, No. 1 are reported studies upon the etiology of measles and scarlet fever; studies of the virus of variola-vaccenia; experiments with de-fatted tubercle bacilli; studies of "detoxicated vaccines"; and the treatment of war gas poisonings, and microphotographic studies of the gonococcus, meningococcus and M. catarrhalis.

There are 114 microphotographs which are truly a work of art, wonderfully well selected and executed and marvelously reproduced.

No. 2 of Volume I describes the preparation of special varieties of culture media, researches on B. pneumosintes and on the bacteriology of the upper respiratory tract in influenza, measles, etc., relating the discovery of six new bacteria; the description of a new

*Diseases of the Skin and Syphilis. By Albert Strickler M.D., Professor of Dermatology and Syphilology, Temple University. Cloth, 689 pages. 214 illustrations. F. A. Davis Co., Philadelphia.

†American Health Congress Series. Vol. III. Paper, 236 pages. National Health Council. New York.

‡Annals of the "Pickett-Thomson" Research Laboratory, Vol. I, Nos. 1 and 2, Paper, 295 pages, numerous microphotographs; Vol. II, No. 1, Paper, 28 pages, 60 microphotographs; Baillere, Tindall, and Cox, London.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

method for the mechanical disintegration of tissues and bacteria; and microphotographic studies of Friedlander's bacillus; influenza bacillus; B. hemophilus hemolyticus; B. pertussis; pneumococcus (all types); enterococcus, and Tunnicliff's diplococcus (measles).

This section contains nearly 100 microphotographs of bacteria exclusive of other illustrations.

Volume II, Part I the discovery of a new bacillus during the study of measles; four new micrococci from the respiratory tract; a study of B. pneumosintes vaccines; and a photographic record of certain chromogenic bacteria.

In the 28 pages of this section there are 60 microphotographs of superlative excellence.

The purpose of this publication is, eventually, to furnish monographs of the various pathogenic bacteria giving an epitome of the various researches upon them, and ultimately to furnish a very complete atlas of bacteria and other microorganisms recording type species photographically.

If this purpose is ultimately achieved much of the difficulty now experienced in visualizing a given germ from a written description will be obviated by reference to the exceedingly valuable microphotographic records now inaugurated in these publications.

It is to be regretted that so valuable a publication should be bound in paper, no other criticism is warranted.

*Annals of the Pickett-Thompson Research Laboratory**

AS THOSE who are familiar with previous volumes of this publication know, its principal purpose is to record photographically the whole range of bacteria and protozoa. The present volume is entirely devoted to researches upon streptococci and constitutes the most comprehensive exposition of this most important, as well as very confused, group of organisms that has, as yet, been presented in one place.

Approximately half the book is devoted to a detailed résumé of numerous technical studies embracing numerous cultural and other methods of study. This portion of the book will be of the greatest interest and value to bacteriologists and laboratory workers as will, especially, the section on the biochemical reactions of the streptococci.

There are also studies of the soluble products of the streptococci, their vitality, their virulence, their immunity reactions, and, among a host of other things, the classification of nonhemolytic types by the use of Crowe's medium.

The final section of the work is embellished by 79 colored plates of reactions on Crowe's medium and 306 microphotographs presented as an aid to the identification of various members of the streptococcus group.

The volume is one of great value and deserves a place in the library of every bacteriologist and laboratory worker.

United Fruit Company Medical Report†

AMERICAN "Big Business" has often been accused of many things in connection with their South American interests. The report in this publication details the very constructive work of the medical department of one of the largest American industries in South America.

The first three sections of the report are concerned largely with statistical data and very informative as to the conditions encountered and the results secured in the company hospitals.

Section IV comprises comments on some of the chief tropical diseases and their treatment.

*Annals of the Pickett-Thomson Research Laboratory, Vol. III. Paper, 316 pages, 306 microphotographs, 79 colored plates. Williams and Wilkins Co., Baltimore.

†United Fruit Company Medical Department Fourteenth Annual Report. Paper, 329 pages. United Fruit Co., Boston.

Section V consists of interesting case reports and Section VI contains twenty-two papers by members of the Medical Staff covering a variety of subjects and investigations. The report is of distinct interest and value.

*A Textbook of Pathology**

THIS fourteenth edition of a well-known text will be welcomed by all those for whom it has long served as a source of reference.

For many years the reviewer has used it as such and has never failed to find some reference to the matter at hand, no matter how unusual or infrequent, and this has doubtless been the experience of many others.

This new edition has undergone a very thorough and comprehensive revision; much new matter covering the important developments of recent years and many new illustrations have been added. The bibliography has also been considerably extended.

The sections covering poisoning and histologic technic in former editions are omitted in this as being better and more fully covered in special works. The technic of postmortem examinations, excluding the preservation and staining of tissues, has been retained.

The book is an excellent example of the printer's craftsmanship and will, undoubtedly, be as favorably received as its predecessor.

Diseases of the Newborn†

IN THIS compact volume the author very ably discusses the diseases which may be encountered during the first month of life.

The book not only represents an intelligent survey of the literature, but ably reflects the practical experience of the author and can be recommended as a most useful reference for both student and practitioner to whom it should come as a very welcome addition to their working library.

Hygiene and Sanitation for Nurses‡

A VERY clearly written textbook for the instruction of nurses which is now in its fifth edition. Eminently satisfactory for the purpose.

The Health of the Child of School Age§

IN THIS small volume are collected a series of ten lectures delivered by various authors upon various subjects before the Institute of Hygiene.

The subjects discussed are: The Dental Problem in Relation to School Children; The Prevention of Nervous Affections in the Young; Diet in Schools; Disorders of Digestion During School Life; The Incidence of Infectious Diseases; Affections of the Nose, Throat, and Ear, and Their Prevention; Eye Troubles; Some Disorders of the Skin and Their Prevention; The Value of Sport and Physical Exercise; and Preventible Deformities in Childhood.

The lecturers are all well-known to the Medical Profession of England and the volume should be of interest and value to school physicians and pediatricians.

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*A Textbook of Pathology. By F. Delafield, M.D. and T. M. Prudden, M.D. Fourteenth Edition revised by F. C. Wood, M.D., Director, Laboratory St. Luke's Hospital, New York. Cloth, 1339 pages, 20 full-page plates, 830 text illustrations. William Wood and Co., New York.

†Diseases of the Newborn: A Textbook for Students and Practitioners. By James Burnet. Cloth, 275 pages. Oxford University Press, New York.

‡Hygiene and Sanitation for Nurses. By G. M. Price, M.D. Cloth, 286 pages. Lea and Febiger, Philadelphia.

§The Health of the Child of School Age. By various authors with a foreword by Sir Thomas Oliver, Professor of Medicine, University of Durham. Cloth 204 pages. Oxford University Press, New York.

*Modern Practice of Pediatrics**

THE book is the most complete one volume work on the subject of Pediatrics that the reviewer has seen and apparently covers as much of the whole field of the subject as could be crowded into one volume.

Besides the author's own views, brief and comprehensive references are given to the important works on most of the various subjects under discussion. Each chapter is well written and there is little in the whole volume that would fail to interest the most critical reader. Particularly noteworthy is the chapter on "Breast Feeding." The author deals with its importance from numerous angles presenting brief and convincing statistics correlated in a most instructive and comprehensive sort of fashion. The chapter on "Metabolism" leaves little that is known on the subject unsaid and briefly states the views of more recent investigators of this subject.

The chapter on "Artificial Feeding" indicates a thorough knowledge on the part of the writer of the important work that has been done in this field. The writer's own views and conclusions are briefly expressed, adequate emphasis being placed on caloric requirements and the proper food elements, for both the normal and the abnormal infant. The author's feeding methods are simple and at the same time based on sound scientific principles.

Obviously the book could not be regarded as an exhaustive treatise on the subject of Pediatrics, but taken as a whole for practical purposes the field is well covered and it is hard to overestimate the value of a reference work of this kind for the student and busy practitioner.

Diseases of the Heart. Their Diagnosis, Prognosis and Treatment by Modern Methods†

WITHIN recent years tremendous strides have been made in the direction of better understanding of cardiac disorders. This progress has been due in great part to the introduction of graphic methods, instruments of precision, into the examination of the cardiac mechanism. The scope of this volume, however, is not confined to a description of the usefulness of graphic methods. It includes within its compass also an account of such knowledge of the diagnosis, prognosis and treatment of cardiac disorders as was in our possession prior to the introduction of these more recent methods.

The author has purposely avoided the discussion of matters of merely theoretical interest. The phraseology is comparatively free from technical terms. The book is written for the clinician, not the specialist, and should find a useful place among the practitioner's books of reference.

A Textbook of Pharmacology‡

THIS is a textbook intended primarily for the student but of value, also, to the practicing physician.

Part I discusses the drugs ordinarily employed in the treatment of disease; Part II is devoted to various new and nonofficial remedies; and Part III includes prescription writing, drug eruptions, incompatibilities, poisons, and antidotes, etc.

The style is succinct and clear-cut. The book should prove of use to the student and a handy source of ready reference for the practitioner.

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*The Modern Practice of Pediatrics. By William Palmer Lucas, M.D., LL.D. Illustrated. Cloth. Pp. 962. The Macmillan Company, 1927.

†Diseases of the Heart. Their Diagnosis, Prognosis, and Treatment by Modern Methods. By Frederick W. Price, M.D., F.R.S. (Edin.) Cloth. Pp. 534. Oxford University Press. American Branch New York.

‡A Textbook of Pharmacology. By A. D. Bush, M.D., Professor of Pharmacology, Emory University, Cloth, 182 pages. P. Blakiston's Son and Co., Philadelphia.

*Ker's Manual of Fevers**

THIS book is intended for the use of English students taking the statutory course of "Fevers" at an Isolation Hospital but may well be added to the working library of physicians at large.

The diseases discussed are: measles, rubella, scarlet fever, smallpox, vaccinia, chickenpox, typhus fever, enteric fever, diphtheria, erysipelas, whooping cough, mumps, and cerebrospinal meningitis.

The text is practical, well-written, and comprehensive within its scope.

As a reference volume for the physician or a text for the student it can be recommended without reserve.

Clinical Diagnosis†

TWENTY years ago Dr. Todd's manual supplied to the student and practicing physician an authoritative source of practical information concerning clinical laboratory methods, their application, and their interpretation.

That it has never relinquished the outstanding position it then assumed and that it is among the most frequently consulted reference books of practically every clinical laboratory are matters of common information. It is not surprising, therefore, that it has passed through many editions and will probably pass through many more, for Todd's manual is one of the books whose inherent excellence and comprehensive usefulness assures its continued existence.

In this new, reset and extensively revised edition the original author is associated with Dr. A. H. Sanford as collaborator whose fitness for such a position requires no comment.

While the revision of the work has been quite general and complete, due to the omission of some little-used methods, and the skilful use of type, the number of pages is not increased although the volume contains more matter than before.

The chapter on animal parasites has been largely rewritten and that on bacteriology extensively revised.

There are 35 new illustrations and many more have been replaced.

As usual, the typography is excellent.

The present manual is quite up-to-date and can be heartily commended without reserve as a necessity for the student, the practicing physician, and the laboratory worker.

Detection of Poisons and Powerful Drugs‡

THAT this work has passed through six editions is a significant intimation as to its worth and usefulness.

The forensic detection of poisons and drugs is a procedure demanding a high degree of skill and experience, as the study of this book amply demonstrates. To those engaged in such work it will serve as an authoritative and complete reference. For the clinical or chemical laboratory worker it should be extremely useful as a source of authoritative methods in the performance of preliminary examinations, Chapter V being a veritable mine of information in this respect.

This volume, like its predecessors, can be recommended without reserve.

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*Ker's Manual of Fevers. Third Edition, revised by C. Rundle, Lecturer on Infectious Diseases, University of Liverpool. Cloth, 6 plates, 15 illustrations, Oxford University Press, New York.

†Clinical Diagnosis by Laboratory Methods. By James Campbell Todd, Ph.B., M.D., Professor of Clinical Pathology, University of Colorado, and Arthur H. Sanford, M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation); Head of Section on Clinical Laboratory, Mayo Clinic. Sixth Edition, Revised and Reset. 748 pages with 346 illustrations 29 in colors. Cloth. Philadelphia and London: W. B. Saunders Company, 1927.

‡Laboratory Manual for the Detection of Poisons and Powerful Drugs. By Wilhelm Autenreith, Sixth American Edition. Authorized Translation by W. H. Warren, Ph.D., Professor of Organic Chemistry, Clark University. Cloth, 698 pages, 60 illustrations. P. Blakiston's Son and Co., Philadelphia.

*Dental Infection and Systemic Diseases**

THE assignment of dental disease to its proper place as a causative factor of systemic disease is a problem the existence of which is recognized, but the solution of which requires a large amount of persistent and systematic study.

To this problem Dr. Haden's little volume brings a contribution of distinct value and definite interest to the physician, the dentist, and the laboratory worker all of whom, perforce, must be alert to the importance of an eventual understanding of this very complicated factor concerned with the diagnosis, treatment, and prophylaxis of disease.

The book records in a connected and succinct way the results of a large amount of research upon dental infections and their relation to disease which has been conducted by the author during the past six years.

After a brief historical résumé the bacteriology of chronic dental infection and the technic suitable for its study are discussed. The most striking feature noted was the very high percentage of positive cultures in radiographic negative teeth. Nine per cent of radiographic positive teeth were sterile, in opposition to the belief of some that all pulpless teeth are infected. The most common organism was the streptococcus, the type determination of which Haden believes unimportant.

The detection of dental infection is discussed. In a large series no information of value was obtained from careful studies of the leucocytes.

The types of systemic disease associated with chronic dental infection are then listed and briefly discussed.

Chapter VI is devoted to a discussion of the lesions in rabbits following the injection of bacteria from chronic periapical infection, and evidence is adduced in support of a tendency on the part of these bacteria to specific localization.

The remainder of the volume is devoted to the history of cases illustrating the relation of dental infection to systemic disease, thirty-five such cases being reported in greater or less detail.

The illustrations and general typography of the volume are excellent and it can be recommended as a definite contribution to the subject.

Recent Advances in Biochemistry†

THE purpose of this little volume is to present as diverse as possible a selection of topics in which the recent advances have been most striking and in which the trend of modern biochemical research is well indicated.

It is not, therefore, a textbook but a review of the more important contributions of recent years to the subject, the selections being necessarily somewhat arbitrary.

The fact that the volume has reached within a year a second edition, suggests that it has been of value to those to whom it is addressed.

The following subjects are discussed in greater or less detail: aspects of protein metabolism; colloids and the physical chemistry proteins, nucleoproteins; the rôle of tyrosine; the carbohydrates; the biochemistry of fats; the rôle of sulphur; the vitamines; hemoglobin and related pigments; the chemical basis of specific immunologic reactions; chemotherapy.

It is obvious by the nature of the volume and the complexity of the subject that their discussion is not exhaustive. As a source of reference of the recent advances in biochemistry it can be recommended as presenting a bird's-eye view of the outstanding contributions of recent times.

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*Dental Infection and Systemic Disease. By R. L. Haden, M.D., Professor, Experimental Medicine, University of Kansas. With a foreword by E. C. Rosenow, M.D. Cloth, 165 pages, 63 engravings. Lea and Febiger, Philadelphia.

†Recent Advances in Biochemistry, John Pryde, Lecturer in Physiological Chemistry, University of Wales, 2nd Edition, Cloth, 379 pages, 38 illustrations. P. Blakiston's Son and Co., Philadelphia.

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EDITORIALS

Tweedledum and Tweedledee

TWO physicians were in earnest consultation over the case of a woman with lobar pneumonia. Her husband was attending the discussion. In the course of conversation the family physician, whom we shall call Dr. N. N. R., remarked that the invalid had been receiving digitol in stated doses. Some minutes later the consultant, let us call him Dr. U. S. P., agreed that digitalis was clearly indicated. The anxious husband eagerly alert paled as he remarked, "But, Doctor, she has had no digitalis whatsoever." It took some time to reassure him to the effect that digitol and digitalis were synonymous terms.

The embryo physician learns his pharmacology and becomes well acquainted with the terms of the United States Pharmacopeia. When he stands diploma in hand, he has not only achieved a doctorate in medicine but he has also mastered a new language, the language of scientific medical terminology which to the uninitiated is as so much Hebrew. He is happy in his knowledge and proud of his acquaintance with the Latin and Greek suffixes and

prefixes which has enabled him to readily understand the derivation of his medical terms.

He opens his office. The corner druggist provides him with "Come to me" prescription blanks. Like as not his first visitors are a long line of detail men representing the various pharmaceutical concerns, each of whom recites his piece with apparent sophistication and with every semblance of scientific accuracy.

Suddenly, like Alice, the young physician finds himself in wonderland. He has placed his sample bottles on a shelf in the cabinet or the laboratory (what physician has not an untidy shelf groaning under a conglomeration of sample medicines?), and in his leisure he decides to study over the day's catch. To his utter amazement he discovers that his linguistic training avails him nothing. Here are words that he has never dreamed of and whose etymologic origin he finds it impossible to trace.

Let's see, what came in today? Thiogen. Oh, yes, something that generates sulphur. Let's read further. A concentrated solution of the sulphates and subsulphates of sodium potassium magnesium and calcium. Ah, this has the ring of familiarity. Sodium sulphate and magnesium sulphate. The old lady has put on her Easter bonnet. What next? Elixir of pyraminal. Literal translation indicates something about fire and amines. No, this is too deep. Let's look farther. Oh, yes, here it is. It contains pyramidon and luminal. The terminologic mutations have been carried one step farther and the mating of pyramidon and luminal has given birth to a new species, pyraminal. But we have been prescribing pyramidon and luminal mixed by the druggist and dispensed in capsules for sometime. Excellent idea to dispense them already mixed. It will save the druggist so much time, the new short name will save the doctor some ink and some brain work and the patient will know that he is receiving the newest and latest of the synthetic preparations. Manufacturer, druggist, doctor, patient, rich man, poor man, beggar man, all will be the happier.

Metatone. That sounds good. An alterative tonic. Let's see what it contains. Vitamin B extract. Here's news. We hadn't realized that they had perfected an extract of vitamins as yet. We live and learn. Nucleic acid. Yes, of course, a derivative from the nuclei of cells which of course must have great tonic value. Oh, yes, and here are a lot of salts, glycerophosphates. Of course that would be tonic and reconstructive. Haven't we a blotter on our desk which assures us that glycerin tonic is excellent? We wonder just what is the difference between glycerophosphate compound and Metatone?

Here's something interesting. Ceanothin. It is indicated as a blood coagulant. Curious our professor of pharmacology was so far behind the times as to have failed to have told us of the great therapeutic value of Ceanothin.

Cheracol. Of course, an alcoholic extract or solution of cherry. The formula is printed on the bottle and it looks good as a cough mixture. There appear to be at least eight ingredients. It would take some time to write up such a prescription using U. S. P. terminology. We can remember the word Cheracol much easier. Yes, let's prescribe Cheracol when the opportunity arises.

Bromionyl with Barbital. Nothing confusing about that. And the ingredients are on the label. Here is a good sensible prescription. Barbital with triple bromide made up into an effervescent granular powder. This should be easy to take. Let's prescribe it. Unfortunately, for this preparation, however, the name is not as euphonious as Cheracol and some days later we have forgotten just how the first word should be spelled. So this does not get prescribed.

What's next? Diasystol. To be sure, this must have something to do with blood pressure but our professor of pharmacology told us positively that no drug had any permanent effect in lowering blood pressure. So, out of courtesy to his memory let's pass this up.

And so the list might go on. The scientific erudition of the detail man is sometimes so great that we wonder why he wastes his time in such an unproductive field. Occasionally in our moments of doubt we wonder how much he is laughing up his sleeve as the saying goes at the avidity with which we appear to drink in his confidential tips for getting better results.

Yes, we are in wonderland. How many preparations of digitalis are there, each with a different name? Exactly what is the pharmacologic difference between digitalis leaves, digifolin, digitalone, digalen, digiglusin and the others? For the life of us we do not know and therefore decide to prescribe that preparation whose name we recall most easily or that preparation which is most attractively packaged or that which for one reason or another seems to be a little easier of administration.

We made so bold within the last month as to write a prescription for 0.1 gram digitalis folia pills. The patient living in a near-by city of ninety thousand inhabitants wrote back with much concern that he had been unable to find a druggist who could fill the prescription. No longer does the mortar and pestle hang above the druggist's entry as a sign of his profession. Too infrequently now does he even use it in the little back cupboard devoted to compounding prescriptions

Within the week we wrote a prescription for tribasic citrocarbonate. A certain manufacturing chemist supplies an excellent effervescent tribasic citrocarbonate, so, to save the druggist trouble, we specified the name of the manufacturer on the prescription. But the word tribasic does not appear upon the ready bottled prescription package and the druggist, a conscientious man, therefore said that he could not fill the prescription. This caution is of course laudable but it is scarcely to his credit that he does not know the exact nature of the ingredients of the ready prepared packages which he is dispensing.

Under how many trade names can you procure a mixture of pyramidon and some phenol-barbital preparation? Wherein lies the superiority of one over the other? Tweedledum and tweedledee, you shut your eyes and take your pick.

Of course, there is something to be said on the other side. If milady prefers to pay a dollar for ten cents worth of rouge put up in a pretty container which will look well on her dressing table, why should her esthetic sense not be also consulted in the matter of tastily prepared medicaments? The manufacturing chemist has accomplished much in the line of making drugs less

disagreeable to take and we owe him a debtor's gratitude. Many of them have also invested great sums in constructive experimental research, much of which is not directly remunerative but some of which ultimately turns out to be of distinct value to the ultimate consumer. This must be paid for out of the income from sales of drugs.

We are living in a day of exaggeration in which unusual claims are made for the most commonplace and in which old friends are scarcely recognized on account of their gaudy apparel and new names. This applies even more to other commodities, soaps, cleaning powders, underclothing, silk stockings, lead pencils, radios, and so on. As long as we are living in a day of exaggerated advertising and copyright names and enjoying it, there is little probability of an early change in the matter of drugs.

We make no plea for a revolution in drug sales methods. We only urge that when a physician prescribes minced pie for his patient whether it be made according to the recipe of Grandma Jones or Old Lady Brown, he knows what are the exact ingredients of said minced pie and how he will expect his patient to react thereto.

—*W. T. V.*

Erratum

In Volume XIII, No. 7, April, 1928, in the article by Christina and Green, entitled "Colloidal Gold Solution," on page 680, third line should read: The indicator is prepared as follows: Dissolve 0.1 gm. of phenol red, etc.